

The University of Chicago

THE RELATION OF HORMONES TO THE DEVELOPMENT
OF COWPER'S AND BARTHOLIN'S GLANDS IN THE
OPOSSUM (DIDELPHYS VIRGINIANA)

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
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THE RELATION OF HORMONES TO THE DEVELOPMENT OF COWPER'S AND BARTHOLIN'S GLANDS IN THE OPOSSUM (*DIDELPHYS VIRGINIANA*)¹

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THREE PLATES (TWENTY-FOUR FIGURES)

I. INTRODUCTION

Experiments inspired by the studies of Lillie ('16, '17) have been conducted in a number of biological workrooms, in this country and abroad, in an attempt to produce an artificial freemartin. Whereas the majority of workers (Greene, Burrill, and Ivy; Dantchakoff; Raynaud; Turner; and others) have utilized the common laboratory mammals — rats, guinea-pigs, and mice — for such experiments, investigators in this laboratory have focused much of their attention on the American opossum (*Didelphys virginiana*). The advantages of studying various phases of sex differentiation in pouch-young born in a sexually undifferentiated state are obvious, and is a condition which is not prevalent in higher mammalian forms. Many of the findings have already been reported (Moore, '39, '41a, b; Moore and Morgan, '42, '43; Morgan, '43; Wheeler, '43) and a number of investigations are still in progress. In the course of these researches, which involved the effects of hormones on sex differentiation, it became evident that Cowper's glands, the only accessory sex glands occurring in the normal development of both sexes, might supply pertinent information to such incompletely answered questions as: (1) When do sex hormones appear in the course of normal development? (2) To what extent are genetic limitations imposed on homologous structures? (3) Are the accessory sex glands of the opossum dependent upon gonad-secreted hormones for their early differentiation? (4) Do homologues of characteristically male accessory sex glands persist as functional units in the adult female?

In order that an accurate analysis might be made of the effects of the various treatments on Cowper's gland and its homologue

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(Bartholin's gland) in the female, it was first necessary to conduct a complete study of the normal development of these structures. The organization of the following presentation is divided into three main sections. Part III deals with an extended account of normal development, Part IV with the effects of administered androgens, estrogens, and gonadotropins, and Part V with the partially completed studies of castration experiments.

The writer wishes to express his gratitude to Prof. Carl R. Moore for suggesting the problem and supervising the course of this research. Grateful acknowledgment is made to Dr. Gregory Stragnell, Schering Corporation, for testosterone ointment, testosterone propionate ointment, and oil solutions of Progynon B; to Dr. George Cartland, Upjohn Company, for gonadogen; to Dr. Donald Wonder, Cutter Laboratories, for gonadin; and to Dr. Oliver Kamm, Parke Davis and Company, for theelin.

II. MATERIALS AND METHODS

Cowper's glands of 166 male and female control and experimental animals have been used in this study. Sample data for a number of these animals are recorded in tables 1 and 2. The glands of a majority of the younger stages have been studied from slides in the collection of Prof. Carl R. Moore. The preparations were standardized 10 micra serial sections generally of the entire posterior one-half of the Bouin-fixed animal, and were stained with Harris' hematoxylin. Included in these preparations were normal and treated stages of known- and estimated-aged animals. The latter, animals of unknown delivery date, were weighed, measured, and classified by comparison with weights and measures of animals of known age which had been arranged in the form of a normal key by Moore and Bodian ('40).

A number of glands of the younger stages and all of the glands of the older stages (102 days through maturity) were dissected from the animals at autopsy, generally along with the muscles and erectile bodies to which they were attached. These were fixed in Bouin's fluid, and successively dehydrated in alcohol, cleared in wintergreen oil, imbedded in paraffin, and serially sectioned at 10 micra. Ehrlich's acid hematoxylin and Heidenhain's modification (Azan) of Mallory's connective tissue stain were used for staining purposes.

The hormones used for the experimental procedure were employed as follows:

TABLE 1

Comparative measurements of normal and treated pouch-young and juvenile male opossums.

SERIAL NUMBER	AGE AT DEATH	SNOUT- RUMP	HEAD LENGTH	WEIGHT	TREATMENT ¹	RELATIVE VOLUMES OF COWPER'S GLAND
Normals						
	(days)	(mm.)	(mm.)	(gm.)	(days)	
16B	5	17.0	7.0	0.37		11.4
2C	10	21.9	8.9	0.82		55.7
33A	16	33.0	10.7	1.35		187.9
10B	24	38.5	14.1	2.58		511.3
34B	32	54.0	19.0	5.67		957.9
87A	44	65.5	23.4	10.00		
246A	65	113.0	36.5	37.00		21293.1
50A	84	135.0	46.0	64.00		
62A	100	155.0	49.5	80.00		
224	125	240.1	71.4	372.00		
176	150		...	370.00		
Androgen-Treated						
96A	16	36.5	12.5	1.87	3-15TO	509.0
63A	22	38.0	12.6	2.00	10-21TPI	2139.7
47A	24	37.0	13.0	1.80	3-24TO	1686.6
64A	31	47.5	16.0	3.89	10-30TPI	4097.2
30A	32	54.0	17.5	5.83	16-31TPI	
65A	39	55.0	19.8	6.00	10-31TPI ²	
98	100	200.0	58.0	168.00	70-99TPI	
Gonadotropin-Treated						
99A	17	34.0	11.5	1.90	10-16Gg	588.5
102A	21	41.0	13.5	2.43	10-20Gg	761.6
112	30	39.5	13.5	1.97	15-29Gd	3438.4
196A	63	98.0	32.4	24.44	54-62Gd	47874.8
201A	70	115.0	36.0	34.00	53-69Gd	
202	80	148.0	47.0	71.00	54-79Gd	
89	100	175.0	52.5	140.00	85-99Gg	

¹ TO, testosterone ointment; TPI, testosterone propionate injected; Gg, gonadogen; Gd, gonadin.

² No treatment after day 31.

Androgens. The synthetic male hormones, testosterone and testosterone propionate, were administered to most of the early pouch-young by inunction in the form of a lanolin base ointment. Some of the early pouch-young, and all of the older-aged specimens received subcutaneous injections of testosterone-propionate. The earliest treatments were initiated on the third day after birth, which is prior to sexual differentiation.

Estrogens. The female hormones were administered in much the same way, that is, by cutaneous applications of ointments containing estradiol and by subcutaneous injections of Progynon B or theelin. The inunction method of administration was initiated as early as day 5, and was used exclusively prior to day 43 except in two cases.

Gonadotropins. The gonadotropic principle of pregnant mare serum was administered by subcutaneous injection of either gonadogen or gonadin. These are commercial preparations of highly effective pituitary-like, gonad-stimulating substances.

A more complete enumeration of dosages and duration of treatment will be found in the text.

The studies of the possible effects of early castration have been carried out on the glands of six male animals. These were castrated by Professor Moore at ages of 22–40 days, and autopsied at subsequent periods in development, between 50 and 102 days.

Measurements of certain dimensions were made either by counting serial sections or by the use of an ocular micrometer. Volumes of the amounts of tissue present were obtained by projecting each section at 200 magnifications, tracing the outlines of the glandular parts, and then retracing these outlines with a planimeter. The volumes are expressed as relative rather than as absolute units (tables 1 and 2).

A word should be said about the terminology employed; in the main it follows the simplified usage of McCrady ('40) and Chase ('39). The term corpus cavernosum will be used to designate the corpus cavernosum penis in the male, and the corpus cavernosum clitoridis in the female. Similarly, the corpus spongiosum refers to the corpus cavernosum urethrae. The terms sagittal urethral plate and urethral plate are used interchangeably, and are synonymous with cloacal septum, urogenital plate, ectodermale Kloakenplatte, and Phallusleiste. The three paired lobes of Cowper's glands, a characteristic feature of male marsupials, are termed Cowper's 1, 2, and 3, representing the largest, intermediate, and smallest lobes, respectively. The female homologue is termed Bartholin's gland or the bulbo-vestibular gland.

III. NORMAL DEVELOPMENT

Keibel ('04) described certain stages in the development of Cowper's gland in *Echidna*, and van den Broek ('08, '10a, b) gave detailed descriptions of a number of early stages in the development of this gland in several species of marsupials, including *Perameles*, *Halmatarus*, and *Didelphys*. Buchanan and Fraser ('18) and Fraser ('19) gave a fairly complete account of the origin and early development of Cowper's

gland in the marsupial, *Trichosurus vulpecula*, but failed to extend their studies to older stages. Recently, Chase ('39) mentioned briefly certain features of the early development of Cowper's gland, and McCrady ('40) described an early stage in the development of both Cowper's gland in the male and its homologue in the female—the latter report also gave a short account of the condition found in an adult female. Other investigators (Cowper, '04; Owen, 1868; Young, 1879; Oudemans, '03; Disselhorst, '04; and Chase, '39) have contributed much to the anatomy of Cowper's gland of the adult marsupials of many species. Among placental mammals, the early stages in the embryological development of Cowper's glands have received attention from several workers—namely, Bissonnette ('24) in the cow; Hall ('36), Siddiqi ('37), and Barnstein and Mossman ('38) in rodents; Müller (1892), Lichtenberg ('06), Felix ('12), and Eggerth ('15) in man. However, in spite of these many investigations, a complete history of the development of this structure had not been described.

The following description of the normal development of Cowper's and Bartholin's glands of the opossum is based on a series of 67 animals: 3 undifferentiated (1–4 days); 33 immature males (5–150 days); 7 adult males; 21 immature females (5–150 days); 3 adult females (2 breeding, 1 anestrus).

COWPER'S GLANDS IN THE MALE

A. Embryonic and juvenile stages

Origin of anlagen to 14 days of pouch life. Cowper's glands anlagen appear for the first time in a 5-day-old pouch-young male (snout-rump length 17 mm., head length 7.0 mm.), corresponding to the period at which the sexes become distinguishable by the histological appearance of the gonads. The primordia consist of two small, solid, bilateral thickenings arising from the cranial end of the solid sagittal urethral plate in its proximal portion. This is approximately the same origin as described by Fraser ('19) for *Trichosurus vulpecula*, in which the primordia appear for the first time in a male (greatest length 16.0 mm., head length 6.5 mm.) as solid outgrowths from the ventro-lateral wall of the urogenital sinus at a point where the free margin of the urethral plate reaches the latter. In later stages the solid urethral plate hollows out and becomes the cavernous portion of the urogenital sinus canal. The origin of Cowper's gland from the urethral plate raises the question as to whether it is an ectodermal or endodermal derivative. This will be discussed, in the light of the embryological findings of other investigators, in another part of this paper.

In an 8-day-old animal the anlagen are more prominent, consisting of paired cell masses, one on each side of the frontal ridge portion of the urethral plate (fig. 2). The frontal ridge portion of the urethral plate becomes the cranial wall of the urethra (McCrary, '40), and accounts for the fact that at a later stage the ducts open into the ventro-lateral aspect of the urogenital sinus.

At 10 days the anlagen have increased in size and extend laterally and somewhat dorsally as solid epithelial cords. The lengthening of the sinus has increased the distance of the gland anlage from the urogenital orifice. The antero-posterior dimension has increased to 0.10 mm. as compared with 0.07 mm. for the 5-day-old animal, while the volume of the anlagen has increased five-fold. Mitotic figures are abundant in all regions.

From day 11 to day 14 the majority of changes occur in connection with associated structures. Intervening between the continually lengthening urogenital sinus and the pubic arch is the mesenchyme of the corpus cavernosum penis, which although present in the younger stages, is clearly discernible as a definite structure for the first time. With the lengthening of the sinus the Cowper's anlagen are conveyed in passive fashion to a more anterior position near the pubic arch cartilages.

The 15th and 16th day. By day 15 the first bud-like branchings originate from the main solid outgrowth. These appear as spherical cell nests in cross sections and occupy a position lateral to the lower urogenital sinus. The branchings are more conspicuous and the paired, solid, epithelial cords are longer in their dorsal extent. The solid cords are composed in their proximal parts of cells similar to the embryonic epithelium of the urogenital sinus lining. These cells are lighter staining than the cells of the basal layer, and are arranged in five to six layers, the most peripheral being in contact with the deep staining basal epithelium. The corpus cavernosum and the crura appear as well defined entities, with which, in later stages, all of the developing lobes are in intimate contact.

Four animals, three estimated as to age and one of known age, were studied for the 16-day stage. This stage differs only slightly from the preceding. In the known-aged animal, the dorsal extensions were more elongate, and the small terminal buds, from the main stem of cells, were directed toward the muscle and connective tissue primordia. The latter were located lateral to and in close contact with the outer wall of the rectum. Thus the 16-day stage foreshadows to some extent the more extensive branching of the Cowper's anlagen to follow at later

stages. Figure 4 shows a section through the region of the Cowper's anlagen in opossum 33A, the known 16-day male.

The 24th day. The 24-day Cowper's gland anlagen represents an important advance in the development of this organ. In this stage one obtains the first clear indication of the splitting of the primordium into the three definitive, paired, bilateral lobes of the adult marsupial. The gland anlagen extend dorsally toward the lateral boundaries of the rectum, at an approximate 45° angle with respect to the point of origin from the urogenital sinus epithelium. The most elongate portion is situated midway between the rectum and the bulb of the corpus cavernosum penis. Cowper's 1, the largest lobe in the adult, consists of a small dorsal branch from the main cord. Cowper's 2, the intermediate lobe, is represented by an even smaller offshot from the main cord, and Cowper's 3 is questionably represented by a limited number of anteriorly directed cell buds, imbedded in a crescent-shaped mesenchymal stroma on the ventral face of the lower urogenital sinus. In the definitive stage Cowper's 3, the smallest of the three lobes, will occupy the most anterior position of the three pairs of glands, whereas Cowper's 2 will be closely associated with the antero-dorsal aspects of the bulbs of the corpus cavernosum. The corpus cavernosum is well developed and the crura occupy a position just anterior to the bulbs of the corpus spongiosum, which are fairly well developed at this stage.

An interesting feature of the 24-day stage is the absence of lumina in all parts of the Cowper's gland anlagen. The main cords are solid and the terminal parts consist of closely joined cells, forming spherical cell nests as seen in transverse sections. Mitotic figures are abundantly distributed throughout. The absence of canalization is of importance in a consideration of treated stages.

The 31st and 32nd day. The glandular anlagen have attained a position just posterior to the pubo-ischaic arch; this is due, in part, to the lengthening of the "embryonic" sinus and, in part, to the growth of the terminal buds. The most anterior portion (Cowper's 3) is composed of cell nests immediately ventral to the well-defined, smooth muscle layer of the urogenital sinus. Mitotic figures are very abundant in the cells of this portion of the gland anlagen. The other two lobes are better developed. At a distance of 0.14 mm. caudad to the most anterior portion of Cowper's 3, the terminal parts of the developing Cowper's 2 are lodged in the base of the mesenchymal structure destined to become the muscle and connective tissue of this lobe. The position of the mesenchymal tissue is between the ventro-lateral wall of the rectum and the medial wall of the bulb of the corpus cavernosum to which it

is closely applied. The prospective duct of Cowper's 2 is a solid elongate cord of cells extending from a lateral position adjacent to the urogenital sinus to the posterior part of the "embryonic" gland capsule. In its antero-posterior dimensions, including the duct, Cowper's 2 measures 0.10 mm. Immediately posterior to Cowper's 2 the dorso-laterally directed duct of Cowper's 1 appears. It extends toward the anterior face of the pear-shaped mass representing the future encasement of the glandular parts of Cowper's 1. This mesenchymal structure is situated on the dorsal aspect of the posterior extent of the ischio-cavernosus muscle, which covers over the bulb of the corpus cavernosum. The epithelial cords of Cowper's 1 do not penetrate beyond the base of the mesenchymal stroma, whereas those of Cowper's 2 penetrate to approximately one-third the length of their respective mesenchymal confines. The antero-posterior dimension of Cowper's 1, including the solid epithelial cord-like prospective duct, measures 0.14 mm. The solid duct anlage extends through the connective tissue of the corpus spongiosum in a convoluted manner, and begins to show slight canalization near the point of origin from the basal layer of the sinus epithelium.

In an estimated 32-day-old animal, opossum 34B, heavier in weight and with a greater crown-rump length than the above 31-day-old animal (opossum 25), the glandular anlagen were better developed, and small lumina appeared in the more distal parts of the thicker and more expansive developing ducts (fig. 9).

Advances of the 31- and 32-day Cowper's glands over the 24-day stage are: (1) a greater degree of branching of the terminal parts; (2) the appearance of canalization in the proximal duct system; and (3) better developed epithelial anlagen representing Cowper's 1, 2 and 3. The canalization of the developing gland proceeds from proximal parts to more peripheral ones.

The 40th through the 44th day. The gland is advanced over the earlier stages in several important features. Opossum 84, a 40-day animal of known age, was found to have a well developed Cowper's 3, the central parts of which were imbedded in a crescent-shaped stromal area, while the anterior parts diverged into laterally situated lobes—the whole enclosing the ventral aspect of the urogenital sinus musculature. The epithelial elements of the smallest lobe are still entirely solid, consisting, in cross section, of irregular cylindrical and slightly elongate cell nests, which are more closely crowded than in the preceding stage. Cowper's 2 is larger and shows a greater degree of differentiation. The duct of this lobe is hollowed throughout most of its extent, and

the lining epithelium is beginning to organize itself into two to three cell layers.

Immediately posterior to the duct of Cowper's 2 lies the enlarged, canalized duct of Cowper's 1. In addition to being larger than the former, the cells of this duct are approaching the tall columnar type, and the entire epithelium is becoming convoluted. This foreshadows the plicated condition of the ducts in older stages. Within the base of the mesenchymal supporting tissue, which is a pear-shaped structure, the duct of Cowper's 1 expands, and the lining epithelium narrows to a double layer of cuboidal cells. From the lateral margins many cords originate which are tubulate proximally where they join the duct basin, but are solid in their distal portions. Mitotic figures are abundant in all portions of the gland. Cowper's 1 exhibits a greater degree of development than either of the other two lobes, and has extended its boundary along the dorso-posterior aspect of the bulb of the corpus cavernosum and the lateral borders of the rectum. Both sets of erectile bodies — the corpus cavernosum, the corpus spongiosum and their respective paired crura — are well developed at this age.

Counts made of the sections show the approximate antero-posterior dimensions of the different lobes to be as follows: Cowper's 3, 360 micra (0.36 mm.); Cowper's 2, 430 micra (0.43 mm.); and Cowper's 1, 520 micra (0.52 mm.). Ocular micrometer measurements of the lobes of the right side of the glandular complex show the greatest medio-lateral dimension of Cowper's 2 to be 0.99 mm., and that of Cowper's 1 to be 1.08 mm. The greatest lateral width of Cowper's 3, across the central part and, therefore, including the bilateral parts of the gland, measures 1.26 mm. Contrary to the condition of bilateral asymmetry described by Müller (1892) in the human foetus, very little size difference was found to exist between the Cowper's glands of the right and left sides of the opossum. This was borne out by volumetric determinations of the relative amounts of tissue present to either side of the urogenital sinus.

At the level of Cowper's 1 the union of the individual ducts of Cowper's 1, 2, and 3, on each side of the urogenital sinus, results in the formation of two common ducts. These ducts are luminal proximally and are in contact with ventro-lateral wall of the sinus, but fail to penetrate the entire mucosal lining. The epithelium of the common duct is stratified, and is more nearly like the epithelium of the more distal ducts than that of the stratified epithelium of the urogenital sinus canal; this applies even to those parts in apposition with the latter region.

The 52nd day. The epithelial lining of the ducts is acquiring the glandular appearance characteristic of the more advanced stages. In certain segments of the duct epithelium more than one cell layer can be seen. The more common condition, however, is for a single cell layer to line the lumen. Cowper's 3 has undergone but little change in its proportions; these are mainly an increased dorso-ventral height and a closer grouping of cell nests, the latter due to increased terminal proliferation. The cylindrical outgrowths are solid, no tubulation having occurred in any of their parts. The connective tissue zone between the bilateral parts of the gland, in its central portion, is thinner than in the 44-day stage. Cowper's 2 retains its position along the medial face of the ischio-cavernosus muscle. Its greatest medio-lateral dimension, exclusive of the duct, has increased to 1.26 mm.; the antero-posterior dimension is 0.61 mm. The elongate cylindrical branches extend over one-half the distance through the connective tissue stroma, and some of the more distal portions are beginning to canalize (fig. 8). Mitotic figures are abundant.

The largest lobe is situated posterior, lateral, and dorsal to Cowper's 2, along the postero-medial border of the crura of the corpus cavernosum penis and dorsal and lateral to the bulb of the corpus spongiosum. The duct is lined by tall columnar cells and penetrates the antero-medial face of the gland. The number of terminal branchings has increased, and each main branch has radiated at its distal end into a number of smaller extensions. The gland has the structure of a retort flask with the broadest portion directed posteriorly and dorsally, and then flattening anteriorly in the region of the duct. The longest medio-lateral dimension of the gland, exclusive of the duct, is approximately 1.26 mm., although the duct is very much longer than that of Cowper's 2. The antero-posterior dimension is 0.57 mm. The smaller dimension, as compared with Cowper's 2, is due to the spherical shape of the larger lobe in contrast to the flattened, elongate shape of Cowper's 2.

The common duct courses through the fibrous portion of the urogenital sinus and opens by way of a small patent orifice into the sinus canal. This is the first evidence of an open communication between the gland and the urogenital sinus canal. Thus, the 52-day gland exhibits four advances over the preceding stage — an increase in size, a more highly developed duct epithelium, patent ducts opening into the urethral canal, and canalization of certain of the peripheral branches.

The 67th day. Advances in structural differentiation at this age are: (1) a plicated condition of the tall columnar epithelium of the ducts dividing the excretory passageways into longitudinal sinuses; (2) the

confluence of the tubules in the central portion of the originally paired parts of Cowper's 3, such that the separate lobes lose their individuality in one part of their antero-posterior extent; and (3) the proximal portions of the branching cylindrical cords of Cowper's 3 are beginning to hollow out.

The 75th to 84th day. By day 75 (opossum 79A) the different lobes of Cowper's gland have acquired well-defined striated muscle capsules which vary in thickness over the different surfaces. These are derived, according to van den Broek ('10a), from the sphincter cloacae musculature. In Cowper's 3, the ventral lobe, the muscular layer is thick ventrally, less dense laterally and practically absent dorsally in the region of confluence across the mid-ventral line. The glandular parts are separated from the smooth muscle of the urogenital sinus by the intervention of a narrow connective tissue band. Posteriorly the muscular capsule of Cowper's 3 is continuous with that of Cowper's 2, as described by Chase ('39). The prospective glandular parts of Cowper's 3 are still solid cords, but the branching of these cords has proceeded to a point where almost the entire stroma has been filled (fig. 5). The most peripheral branches of Cowper's 1 and 2 are also solid. The antero-posterior dimensions of the three lobes are: for Cowper's 3, 0.58 mm.; for Cowper's 2, 0.95 mm.; and for Cowper's 1, 0.91 mm. Ocular micrometer measurements of the maximal width of Cowper's 3, in the region of confluence, is 2.25 mm., the maximal medio-lateral length of Cowper's 2 is 1.53 mm., and of Cowper's 1, 2.19 mm. (exclusive of ducts in last two lobes).

Eight to 9 days later the solid cords of cells of Cowper's 3, which prior to this age (84 days) failed to exhibit any signs of hollowing, are becoming tubulate. The late date at which this process of canalization occurs in the normal development of Cowper's 3 is of importance in comparison with treated stages.

The 100th day. The 100-day-old Cowper's gland complex (opossum 62A) has acquired certain distinctive characteristics. The most conspicuous of these is the degree of differentiation in Cowper's 3. Whereas this portion of the gland showed signs of hollowing in its proximal parts by day 84, it is now tubulate throughout. The tubules are narrow anteriorly and wider posteriorly in the region of the ducts. The epithelium has the appearance of a true glandular lining layer, and the lumina are partially filled with a light-staining, homogeneous material. In a few of the tubules a number of dark bodies, which on closer examination appear to be mixed leukocytes and degenerate cells from the lining epithelium, are enmeshed in this homogeneous matrix. The

nature of the cellular debris can only be surmised, but a study of treated animals seems to indicate a relationship between the appearance of these sloughed-off cells and leukocytes and the onset of sex hormone production in the animal.

In addition to the changed histological appearance of the glandular components growth changes are also evidenced. The anterior limits of Cowper's 3 have been extended. The gland is, in this region, distinctly bilateral and located on the outside of the ventro-lateral aspects of the urogenital sinus musculature. Proximally these independent portions merge across the mid-ventral line, and the tubules of opposite sides coalesce. Cowper's 1 and 2 also show changes mainly in the extent of the hollowed out cylindrical branches, and in the alignment of the cells which border the lumina forming a single layered epithelial sheath. The individual ducts have increased in degree of complexity of structure and coiling. The common ducts are large, tubular, and lined by the same type of epithelium as found in the glandular parts proper. The lumen of the common duct is partitioned into several longitudinal sections by folding of the columnar epithelium. After traversing the corpus spongiosum in an oblique, medio-caudad direction, the ducts contact the lower urogenital sinus canal mucosa, penetrate it again obliquely, and course posteriorly within the canal as papillae-like structures.

Before continuing it may be advantageous to summarize briefly the development of Cowper's gland prior to the next stage — namely, the 125th day. From the first appearance of Cowper's anlage up to and including the 100-day stage, development is a gradual process. Following the initial branching of each of the paired bilaterally arranged anlagen into the three component parts (between day 24 and day 32), growth is relatively evenly graded in each lobe. Prior to day 83, Cowper's 1 (the largest of the three lobes) is differentiating more rapidly than Cowper's 2 (the intermediate lobe), especially with respect to cavitation of the solid cords. Cowper's 2 is, in turn, more advanced than the smallest lobe, Cowper's 3, although all glandular anlagen are increased in size and in structural complexity at successive age levels. Subsequent to and including the 84-day stages, Cowper's 3, which had previously been lagging in development (utilizing the degree of cavitation as a criterion), suddenly acquired extensive tubulate areas, notably so in the proximal regions. By day 100 the lumina have extended their boundaries to more distal parts, and Cowper's 3 appears more advanced than either Cowper's 2 or Cowper's 1 in two respects

— namely, in the more extensive tubulate condition and in the degree of histological differentiation of the epithelium.

The 125th day. The period from day 100 to day 125 is a critical period in the growth of the paired lobes of Cowper's gland complex. Unlike the gradual developmental processes observed previously, this period is characterized by a very rapid growth phase. The epithelial tissue has increased in amount and the intertubular tissue has been relatively reduced in extent. In cross sections the tubules of Cowper's 1 and 2 are spherical, consisting in more distal parts of a two- or three-layered epithelial wall surrounding a small lumen. Proximally the tubules widen, acquire a single-layered lining epithelium, and become irregular in shape. In longitudinal sections the epithelial parts appear as long cord-like structures radiating fan-wise from the narrowed base of the gland. The lumina are seen as narrow slits in the center of the cords. Many side branches originate from the main cords giving to the whole the typical compound tubular structure of the definitive gland. The epithelium of Cowper's 2 is acquiring a glandular appearance not yet apparent in Cowper's 1. However, the most conspicuous advance is noted in Cowper's 3, where the gland has not only acquired a high columnar epithelium, but exhibits evidence of secretory activity in having a light-staining homogeneous colloid-like material within many of the lumina.

The cords of Cowper's 1 and 2 open into large excretory ducts, the cavities of which are divided into many lumina by glandular epithelial partitions. Adjacent to the gland the epithelium of the ducts is high columnar, but this gradually passes into the low cuboidal type and persists as such until the ducts of all three lobes unite to form the common duct. Within the paired, bilateral, common ducts the epithelium once again becomes tall columnar, a condition which may persist to the mucosa of the urogenital sinus canal.

The nature of the duct relation to the urogenital sinus is variable. In some male animals the ducts open into the urogenital sinus canal without coursing in the canal itself. In other cases the ducts open only after coursing, for a short distance, posteriorly within the canal as papillary structures.

The 150th day. The 150-day Cowper's gland is advanced over the 125-day gland only in size. The tubules are slightly larger in all lobes; the lumina of Cowper's 3 are widest, those of Cowper's 2 are next in order, and are followed by Cowper's 1 with the smallest. The ducts are widely cavitae and partitioned into smaller and larger chambers. The epithelium within the duct varies from tall columnar to low cuboidal

cells with nuclei which are correspondingly elongate or oval, respectively. Figures 19 and 18 show the epithelium of Cowper's 1 and 3 under high magnification.

The 240th day. Only a portion of the glandular material of the 240-day stage was available for study. Microscopic examination of the incomplete glandular areas indicates a condition midway between the 150-day stage and the adult condition which is attained at about 1 year of life. The lumina contain secretion products of a homogeneous nature, and the epithelial partitions are flattened as a result of distention. The adult state is attained by further increase in size of the glandular lobes and greater luminal distention. A number of stages between days 150 and the adult period are desirable for future study.

B. The adult stage

Chase ('39) working in this laboratory, has conducted a thorough investigation of the anatomy, histology and physiology of the adult male reproductive tract in the opossum. Included in the study were Cowper's glands obtained from eight control animals sacrificed at intervals from late November to early June. During the period covered by the study it was found that the glandular parts of each lobe varied in weight and histological structure from the fall of the year to the spring and summer. The histological picture of the epithelial elements varied also during the same period of the year depending upon the amount of secretion present, and the resultant distention of the lumina. In general, the glands were smaller in the fall and larger in the spring of the year, although secretion products were found in the lumina throughout the period covered in the study.

The histological findings in a study of Cowper's glands taken from seven adult males during the months of June, July, and August agree in most respects with the descriptions advanced by Chase. In all cases the largest lobe, Cowper's 1, consists of large, irregularly spherical tubules filled with a homogeneous-staining secretion. The cellular elements of the glandular epithelium are extremely low cuboidal with small flattened nuclei which almost fill the cell body. Between the closely packed tubules a very narrow zone of connective tissue intervenes. Cowper's 2 has less regularly arranged tubules and a highly convoluted epithelium, the cells of which are mainly medium columnar with basally situated, elongate nuclei directed along the long axis of the cell. Within the lumina a grainy secretion is visible which differs in consistency from that observed in the tubules of the larger lobe. The intertubular stroma of Cowper's 2 is broader than in Cowper's 1. About the glandular

peripheral parts is a narrow zone of connective tissue which varies in thickness in different portions along the surface, and enclosing this is a striated muscle coat also of variable thickness along the circumference. The muscle coat of Cowper's 2 is thicker than that of Cowper's 1.

The glandular parts of Cowper's 3, the smallest lobe, are similar to those of Cowper's 2. The elongate tubules are irregular in shape and lined by medium to tall columnar epithelial cells forming low folds which project into the lumina. The intertubular tissue is most abundant in this lobe. The largest lumina were found in Cowper's 1 of an animal sacrificed in early June. Weights of the various lobes were not recorded, but from Chase's data it is evidenced that in almost all cases Cowper's 1 is heavier than the combined weight of Cowper's 2 and 3 by as much as four to five times. The ducts of all parts were extremely large, lined by glandular epithelium of the same nature as the lobe from which they conduct the secretion, and were enclosed in a connective tissue and striated muscular capsule of considerable thickness.

One unusual case was discovered, in conducting an autopsy, which serves to corroborate the lower urogenital sinus origin of Cowper's gland. A male animal with normal-appearing scrotal testes was found to lack a penis and, therefore, the entire cavernous urethra as well. A probe inserted into the external urogenital sinus orifice entered directly into the membranous portion of the sinus. Careful examination of the interior failed to reveal any trace of Cowper's glands. Histological examination of sections of the testes and epididymides revealed normal structural components and mature spermatozoa in both. This is undoubtedly a case of the developmental absence of the cavernous urethra and of the parts of the reproductive tract derived from it.

COWPER'S GLANDS HOMOLOGUE IN THE FEMALE

It was judged to be important to determine to what extent Bartholin's gland of the female resembled the homologous male structure, both in the mode of development and in the degree of definitive morphological complexity. Then, too, Bartholin's gland is the only accessory sex gland normally present in the female opossum — the prostate homologue (Skene's glands) never occurring in the natural course of sex differentiation (Moore, '39, '41a). Dependent upon such considerations was the ultimate interpretation of the reactions of this gland to experimental treatment and their probable bearing on the problems of normal sex differentiation.

No one, as far as the writer is aware, has ever traced the gland from its first appearance to the adult condition in any mammal. This was made possible in the present study through the availability of a graded series of female pouch young and subsequent stages, including juveniles and several adults. Some of the animals studied are listed along with other informative data in table 2.

TABLE 2

Comparative measurements of normal and treated pouch-young and juvenile female opossums.

SERIAL NUMBER	AGE AT DEATH	SNOUT- RUMP	HEAD LENGTH	WEIGHT	TREATMENT ¹	RELATIVE VOLUMES OF BARTHOLIN'S GLAND
Normals						
	(days)	(mm.)	(mm.)	(gm.)	(days)	
1A	5	16.3	6.3	0.39		10.8
2B	10	21.9	8.9	0.82		16.5
33B	16	32.0	11.0	1.38		43.4
10A	24	38.5	14.1	2.58		119.2
34A	32	53.0	17.9	5.22		299.8
87B	44	70.0	24.5	11.35		
83B	67	96.0	32.5	24.30		
50B	84	140.0	43.0	62.00		
62B	100	160.0	51.1	100.00		
228	125	245.0	74.7	320.00		
178*	150		...	410.00		
Androgen-Treated						
96B	16	36.5	12.5	1.87	3-15TO	425.8
63B	22	38.0	12.2	1.82	10-21TPI	1685.3
47B	24	38.5	13.0	1.90	3-24TO	1534.9
64B	31	47.0	15.5	3.47	10-30TPI	3397.2
30B	32	50.0	16.1	4.75	16-31TPI	
65B	39	52.0	17.8	4.79	10-31TPI ²	
117	95	221.0	65.2	216.00	3-15TO; 16-93TPI	
Gonadotropin-Treated						
99B	17	37.0	11.6	2.00	10-16Gg	78.2
102B	21	41.2	13.5	2.58	10-20Gg	117.2
113	30	43.5	15.2	2.93	15-29Gd	256.7
196B	63	95.0	32.3	23.75	54-62Gd	
201B	70	115.0	37.0	36.00	53-69Gd	
205	80	196.0	46.0	78.00	54-79Gd	
90	100	173.0	52.0	130.00	85-99Gg	

¹ TO, testosterone ointment; TPI, testosterone propionate injected; Gg, gonadogen; Gd, gonadin.

² No treatment after day 31.

A. Embryonic and juvenile stages

The 5th to 10th day. As already described in the discussion of the male Cowper's gland, the first anlagen of Bartholin's gland in the female opossum were observed at 5 days of age. The primordia are visible as bilateral, rounded projections extending laterally from the basal layer of the sagittal urethral plate in its cranial portion, about 0.04 mm. anterior to the opening of the urogenital sinus into the uroproctodeum (fig. 1). The basal layer of the sagittal urethral plate is a well-defined peripheral layer with large darkly-staining nuclei and consists of regularly arranged columnar cells. This layer is also the peripheral layer of the evaginated Bartholin's anlagen, and, as in the male, is continuous with it. The anlagen are solid structures made up entirely of embryonic cells which do not differ histologically from the cells of the solid urethral plate. Mitoses are abundant mainly in the basal layer. Two days later, in opossum 12B, the anlagen were found to lie 0.25 mm. from the urogenital sinus orifice as a result of the lengthening of the urethral plate.

The 14th and 16th days. The anlagen appear just posterior to the pubic cartilages, close to the union of the upper and lower urogenital sinus division. The early solid demilune-like appearance is transforming into a solid linear extension at right angles to the urethral plate. By day 16, in cross sections anterior to the point of origin of the primordia, small isolated spherical cell nests (branches of the main stem) are visible lateral to the urogenital sinus. The peripheral branches lie in the mesenchymal stroma outside the musculature of the sinus and dorsal to the corpus cavernosum. The latter is situated ventral to the urogenital sinus as a crescent-shaped structure. Volumetric measurements (table 2) of the 5-, 10-, and 16-day stages are in the order of 10 to 20 to 43, or more clearly, the anlagen have doubled their volume by 10 days and more than quadrupled it by 16 days from the time of origin. However, the male anlagen of the same age are more than four times the volume of the 16-day female anlagen.

The 24th day. It will be recalled that the first signs of morphological differentiation occur in the 24-day-old male opossum in the appearance of rudiments of the large lobe, Cowper's 1. If we consider the first major difference between the development of the male and female Cowper's gland anlagen to be a growth difference (table 2) apparent at very early stages, then the second difference of importance is the absence, in the female, of the morphological differentiation into lobes such as occurs by day 24 in the male.

Bartholin's glands of the 24-day female are still solid cords with peripheral branchings of small magnitude. The inner core of the solid cords, which extend from the outer epithelial layer of the urogenital sinus, are similar to those lining the inner wall of the urogenital sinus. The cytoplasm is very light and the nuclei large and elliptical in shape. In the distal and peripheral areas the anlagen are composed of columnar cells with elongate nuclei, the long axes of which parallel the long axes of the cells. The anterior portions of the branched cord are imbedded in a dense mesenchymal stroma which surrounds the urogenital sinus. The volume of tissue present is 119.2 as compared with 511.3 in a littermate male of the same age.

The 31st and 32nd days. In three animals studied the condition was noted to be similar. A number of small terminal buds originate in the anterior region of the primordium and occupy a position lateral to the ventral half of the urogenital sinus (fig. 3). The entire anlagen, including the main stem originating from the urogenital sinus epithelium, is enclosed in a well-defined mesenchymal mass, the forerunner of the connective tissue and muscular capsule of the definitive gland. All parts of the primordium, except for one small sector next to the main cord, are solid cellular areas. In the 32-day-old female (opossum 34A) the most proximal parts of the solid cord begin to show signs of hollowing out, as indicated by the presence of a small tubulate section. Mitotic figures are abundantly scattered throughout the various parts. The corpus cavernosum is well defined and lies ventral to the urethra with the bulbs arching dorsally toward the pubic cartilages. In general no very marked advance over earlier stages is noted aside from the small canalized area mentioned above. The 32-day anlagen has a tissue volume of 299.8 as compared with 119.2 in the 24-day stage. In the equivalent-aged littermate male, opossum 34B, the volume of the anlagen was 957.9, or approximately 219% greater.

The 44th day. In the interim from day 32 to day 44, the female Cowper's gland, as viewed in cross section, continues to branch at its terminal end into a number of small spherical cell masses. The terminal parts are non-tubulate, but as the point of origin is approached the enlarged, heavy, cellular cords in contact with the urogenital sinus epithelium acquire lumina, some of which contain a homogeneous light-staining material — probably a cell exudate of the surrounding epithelium. The luminal parts penetrate several cell layers of the urethral musoca, but fail to reach the urethral canal. The gland is confined to the ventro-lateral aspect of the urogenital sinus, surrounded by, and

imbedded in, an abundant loose mesenchymal stroma just dorsal to the corpus cavernosum clitoridis.

The 67th day. Between day 44 and day 67 the paired gland has branched into a number of terminal shoots foreshadowing the compound tubular arrangement of the adult gland. In cross section these branched parts appear as a number of rounded clusters of cells, separated by the dense connective tissue in which they are imbedded. The whole is enclosed in a striated muscular coat which is first becoming a histologically recognizable layer. In position the glands lie one to each side of the mid-ventral line between the corpus cavernosum clitoridis and the urethra. They are closely applied through their muscular capsules to the muscle of the latter and by connective tissue to the former. The branched terminal cords of the gland, some of which are tubulate, join posteriorly to form larger cords of cells, which unite with a broadly cavitate duct lined with several stratified layers of convoluted epithelium. This duct penetrates the peripheral layers of the urethral mucosa in an obliquely posterior direction, acquires a short zone of lining epithelium characteristic of the urethral mucosa, and opens into the urogenital sinus canal by way of minute openings. In this animal, opossum 83B, the ducts opened on papillary structures which invaded the sinus canal and projected within the lumen for a short distance posteriorly. The condition is less usual in females, but encountered very generally in males. There is no evidence of differentiation into the lobes characteristic of the male Cowper's gland; instead, the bilateral Bartholin's glands are localized to each side of the ventrolateral aspects of the urogenital sinus. The antero-posterior dimension of the gland at this age is 0.81 mm., compared with 0.15 mm. in the 24-day female.

The 75th day. Growth continues, and by day 75 the glands have increased their antero-posterior dimensions to 1.23 mm. (opossum 79B) — a condition which has carried them beyond the proximal part of the median corpus cavernosum to a point dorsal to the paired crura. A new feature in the glandular development occurs in the form of ventrolateral branchings. These are, at this stage, relatively inconspicuous small lateral outgrowths in the anterior region directed away from the main body of the gland. The terminal parts are as yet solid cell clusters in cross section, but more compactly arranged than in the preceding age level, which indicates further branching of the terminal parts. More posteriorly the larger cords hollow out and form tubular areas lined by one to two layers of cuboidal epithelium.

The 83rd and 84th days. The 83- and 84-day gland is markedly advanced over the 75-day stage. The majority of distal tubules are luminal and much more closely allied, which indicates that the structure is gradually growing and differentiating. The luminal tubules are more conspicuous in the central parts of the gland where crowding occurs; peripherally the tubules are less close together. The striated muscle capsule is better developed and fairly thick on the lateral, medial, and dorsal surfaces of the gland. The ventral aspect is in contact with the dorsal surface of the corpus cavernosus body. Proximally the terminal tubules coalesce to form larger tubules of irregular configurations. The lining epithelium is generally tall columnar with spherical or slightly elongate nuclei occupying the proximal two-thirds of the cell. Each nucleus contains one large nucleolus and several smaller chromatin clumps. The glandular elements gradually diminish proximally, near the point of their origin, and large tubular ducts with convoluted epithelium extend ventro-posteriorly toward the ventral part of the lower urogenital sinus. The antero-posterior dimension of the 84-day gland (opossum 50B) was 1.01 mm., and that of the 83-day gland (opossum 190) was 1.78 mm.

The 100th day. At 100 days the glandular epithelium of the bulbo-vestibular gland is in an advanced state of differentiation, but varies regionally. Centrally the lumina are large and bounded by tall columnar cells; more peripherally the lumina are smaller and enclosed by medium columnar cells; in the outermost areas, both medially and laterally, the tubules are still solid or else but slightly hollowed out (fig. 13). A gradient is also present from anterior to posterior levels of the gland. Thus, anteriorly the smaller branches are solid or very slightly luminal. Centrally the greatest degree of differentiation is noted; here the lumina are large, densely packed, and with very little intertubular connective tissue. Posteriorly the glandular elements retain the well developed character of the central parts to the most posterior regions of the gland. This is an expected difference inasmuch as the gland originates in that region which remains as the posterior portion of its structure, whereas the anterior parts represent the most terminal, hence the youngest, areas to develop. The duct is short and lined by the same type of glandular epithelium as that found in the gland. It courses from the medial aspect of the gland near its posterior end, parallels the basal layer of the urethral mucosa for a short distance, then penetrates the squamous epithelial layers, and opens into the urogenital sinus canal. The exact location at which the ducts open varies from a ventro-lateral to a mid-lateral position in the different animals

studied; the position is not as rigidly fixed as in the male. The epithelial character of the glandular parts proper and their ducts (one on each side of the urogenital sinus) is most comparable to the condition in the smallest lobe (Cowper's 3) of the male at this same age in development. However, the female condition with respect to epithelial differentiation is further advanced than that observed in any of the male parts. The antero-posterior dimension of the gland varies from 1.21 mm. in opossum 62B to 1.71 mm. in opossum 82B.

In addition to the advanced condition of the glandular epithelium, the connective tissue and muscular elements are also more conspicuous. The striated muscular capsule is heaviest on the lateral aspects of the gland, and relatively thin dorsally and in the region of the smooth muscle of the urethra. In the latter area connective tissue, smooth muscle fibers, and glandular elements intermingle to some extent. In the medio-dorsal aspect of the gland, where it encloses the urethral musculature, there is a complete absence of striated muscle. In the larger of the two Bartholin's glands (opossum 82B) the accessory diverticulae, budding from the lateral aspect of the anterior region of the gland as mentioned in the 75-day stage, are considerably enlarged (fig. 13). Another feature which can be seen in figure 13 is the contiguity of the bilateral glands in the region ventral to the urogenital sinus. It has been observed in studies of serial sections that occasional coalescence between tubules of opposite sides will occur in the areas of loose connective tissue which act as an incomplete partition between the paired structures. Prior to 100 days this fusion of tubules across the mid-line has not been observed, although the bilateral parts approach each other to a greater degree at successive age levels. Subsequent to 100 days the confluence of originally bilateral parts in a broad zone midway between the cranial and caudal limits becomes a permanent feature of the bulbo-vestibular gland. It should be recalled that the bilateral parts of Cowper's 3 in the 67-day male also meet across the mid-ventral aspect of the sinus musculature. This antedates the same condition in the female by fully 30 days. That this implies a more immediate relationship between the female homologue and Cowper's 3 than between the former and the other lobes of the male gland is entirely probable. It is known, of course, that in the female rat the homologue of the male prostate gland, when well developed, corresponds in histological appearance to the ventral lobe only (Price, '42).

The 125th day. Morgan ('43; Moore and Morgan, '43) has shown that ovarian activity in the opossum, as judged by antrum formation, is clearly demonstrable for the first time at 125 days. Wheeler ('43)

reported a marked activity of the basophil elements of the anterior lobe of the pituitary gland in males and females between days 101 and 104, and a secretory phase with resultant degranulation and depletion of the stored colloid. It was, therefore, expected that some effect might be registered upon the bulbo-vestibular glands subsequent to the 100-day period.

Two animals, opossums 228 and 229, were available for a study of the 125-day stage in the development of the glands. In both, the glandular epithelium of the well-defined tubules was mainly tall columnar, the intertubular, reticular connective tissue very narrow, and the entire gland disposed around the ventro-lateral aspect of the lower urogenital sinus. In such respects the gland resembled the glands of earlier stages of development. The most obvious difference, however, was the considerable desquamation of cells into the lumina of the larger tubules along with an infiltration of leukocytes and other undetermined elements. Such changes were most conspicuous in the gland of opossum 228.

In sections prepared with Heidenhain's modification of Mallory's connective tissue stain (Azan) a number of distinctly different elements were identified in the heterogeneous, cellular conglomerate within the lumina. Under high magnification these were separable into the following: (1) medium-sized cells with a granular, light-staining cytoplasm and several small bright red nuclei, obviously polymorphonuclear leukocytes; (2) small and medium-sized basophilic cells, the former with large, deep red nuclei, and the latter with large, deep-staining blue nuclei and vacuolated cytoplasm; and (3) large cells with pale cytoplasm and deeply-stained pyknotic nuclei, undoubtedly degenerate cells of the lining epithelium. Stages of cellular extrusion into the tubules were manifest in the form of cell nests, composed of a number of clumped large cells, pinching away from the lining layer of epithelium. Very little matrix was present about the free cells in the lumina. The most extensively desquamated areas occurred in the middle part of the gland, that is, midway between the anterior and posterior ends. Even in this region, however, contiguous lumina were found, some of which contained large amounts of cellular debris, others contained lesser amounts, and still others none at all. Another observation which arouses some speculation is the presence, among the single-layered lining epithelium, of sporadically occurring cells which have basophilic granules in the cytoplasm. These cells are relatively few in number and suggest gland cells with secretion antecedents. Cytological studies are much to be desired in a clarification of these observations.

The antero-posterior dimension of the glands in opossums 228 and 229 were 2.92 mm. and 2.73 mm., respectively. Thus, while the character of the glandular epithelium, exclusive of the desquamation, has not been modified in comparison with the 100-day-old bulbo-vestibular gland, the size of the gland has increased noticeably. The onset of ovarian secretion has not, therefore, inhibited the continued growth of the gland. In fact, the smaller of the above measurements shows an increase of 60% over the largest, and 126% over the smallest, of the 100-day-old glands, whereas, the larger of the two measurements is, for the same comparisons, 71% and 141% greater than the 100-day values. The leukocytic infiltration and the sloughing of cells is a condition met with in treated animals as well as untreated, and will be discussed more fully in another section of this paper.

The 150th day. Bartholin's glands at 150 days are similar in appearance to those of 125 days. The areas in which the sloughing off process is most extensive has remained within the central part of the gland, more especially around the urethral region. Anteriorly, peripherally, and in the posterior parts, very few lumina were observed to contain any cellular debris (fig. 20). The reason for this selective condition may in some manner be correlated with the more closely packed cells of the high columnar epithelium found in the central portion of the gland. The antero-posterior dimension of the gland is 3.40 mm. on the left side and 3.00 mm. on the right side. Direct measurements of the sagittal sections of opossum 178 show the greatest antero-posterior dimension to be 3.53 mm., a value which compares very favorably with section counts in opossum 179. The shape of the gland may be likened to a bilaterally arranged semi-round structure with an outer convex surface and an inner slightly-concave surface which cups the urethra. These are separated by a median dorso-ventral connective tissue partition, except for an area midway between the anterior and the posterior extremities where the glandular parts coalesce (fig. 20). Accessory lobes, larger than those seen in the 125-day stage, extend laterally from the main body of the anterior region of the gland. No sloughing of cells occurs in the lumina of these accessory diverticulae.

The connective and muscular tissue elements of the 150-day bulbo-vestibular gland are somewhat increased in comparison with the 125-day stage. The former has increased considerably between the individual tubules and also around a number of adjacent tubules. Dense wavy reticular fibers run through the looser stroma and more or less partition the glandular parts into local areas. The striated muscular capsule, unlike the connective tissue, is not continuous over the entire

periphery of the gland. In the most anterior parts the striated muscle capsule forms a complete covering about the inner connective tissue layer. Just posterior to this region the muscle layer is absent from the ventral aspect of the gland, so that the connective tissue of the gland is directly continuous with the dorsal surface of the corpus cavernosum clitoridis. In the horseshoe-shaped area of tubular confluence the muscle coat is present only on the broad free lateral aspects of the gland. The dorsal aspect is separated from the urethral musculature by only the connective tissue. In this region smooth muscle fibers are seen among those tubules of the gland which are closest to the urethral musculature. This condition has been described in the human (Maximow and Bloom, '41). Striated muscle fibers also penetrate the intertubular tissue of the peripheral regions, next to the lateral aspects of the gland.

Summarizing the development of this structure up to the adult stage, the most salient features are: (1) an origin similar in source and position, and at a similar age to that described for the male; (2) a slower rate of development than in the male (which is already measureable at 10 days); (3) the lack of lobe formation such as occurs in the male, and the persistence, along the ventral and lateral aspects, of the urogenital sinus as a localized organ; (4) a very gradual growth in size up to 100 days; (5) a better advanced glandular epithelial structure at 100 days than in a correspondingly aged male; (6) the conspicuous desquamation reaction at 125 days which correlates with the first signs of ovarian activity; and (7) the confluence of bilateral parts across the mid-ventral line as in Cowper's 3 of the male, but occurring at a much later age.

B. The adult bulbo-vestibular gland

Two distinct phases in the reproductive cycle of the female occur (in the laboratory) during the year — the breeding period from February to June, and an anestrus period from July to January. Three females sacrificed in February, May, and September, respectively, provided the material for a study of the adult bulbo-vestibular glands during these periods.

The anestrus gland. The anestrus vestibular gland, obtained from a normal, healthy 5½ pound adult female sacrificed in September, is a morphologically well-defined structure. In position it is localized along the ventro-lateral aspects of the urethra, with the dorsal concave aspect of the gland closely applied to the urethral musculature. Histological studies, of representative sections, show well-developed, large,

closely wedged tubules with a high columnar epithelium, as shown in figure 23. Through the broadest portion of the gland, in the confluent area where the bilateral glands merge across the mid-line, the connective tissue capsule fibers penetrate among the tubules and divide them into lobular units. As described in younger stages, the smooth muscle of the urethra invades the intertubular spaces along the urethral aspect. Ventrally the thick connective tissue capsule is encased by striated muscle. Laterally the muscular coat merges in the dorsal region with smooth muscle fibers extending from the urethra. Desquamation of cells is still evident, but very limited as compared with the 150-day stage. In some lumina a light-staining matrix is observed, the exact nature of which is uncertain. One fact is clearly indicated — namely, that the gland is larger and better developed histologically than at younger stages.

The early breeding gland. The condition of the urethral epithelium in the female sacrificed in February indicated a heightened period of ovarian secretory activity. Microscopic examination of the bulbo-vestibular gland revealed an arrangement essentially similar to the anestrus gland. The tubules were, however, a bit more closely packed, and the intertubular connective tissue less conspicuous. Most of the cells in the central portion of the glandular epithelium were medium to tall columnar; peripherally, cuboidal epithelium was prevalent. Large sized blood vessels were found penetrating the intertubular tissue, and certain lumina contained a colloid-like material. Other tubules were filled with desquamated epithelial cells such as were noted in the anestrus opossum. Functionally the gland appeared active rather than quiescent. Measurements of the antero-posterior dimension of the bulbo-vestibular gland indicate an approximate value of over 6.0 mm. This represents a 100% size increase over the 150-day gland.

The late breeding gland. Bartholin's gland presents an interesting histological condition in the animal sacrificed in May, during the latter part of the breeding season. The gland is composed of closely packed tubules almost to the exclusion of all other elements. Only a very thin band of reticular connective tissue fibers is interposed between the single layered epithelial tubules (fig. 22). The epithelium is predominantly tall columnar with other types being sparsely represented. The lumina are irregular in cross section and contain but very slight evidence of desquamated cells; instead, there is marked evidence of heightened secretory activity. Certain lumina contain an abundant amount of colloid which stains deeply with Azan; others are devoid of any secretion product. The gland is more highly vascularized than in the

early part of the breeding season. In cross section the gland has the same general shape as a sagittally cut surface of a kidney. The part which would correspond to the hilus faces dorsally and is invaded by a ventral extension of the smooth muscle and connective tissue of the corpus spongiosum, except in the region of tubular confluence. In the region of tubular coalescence the dorso-ventral dimension of the gland is 3.50 mm. The gland is also widest in this zone, measuring 7.00 mm. from one lateral border to the other. The antero-posterior dimension is, as in the February gland, 6.00 mm. The entire gland is surrounded by a thick connective tissue layer which invades the inner parts as the intertubular stroma.

Comparatively, the bulbo-vestibular gland of the latter part of the breeding season is more highly developed than the gland of the early breeding season. There appears to be a gradual development from the anestrus condition to the fully developed breeding period structure. While it might be inferred that the estrogenic secretions in the adult female opossum seem to stimulate the bulbo-vestibular gland, a more plausible suggestion will be advanced in considering the experimental findings.

A comparison of the adult male and female glands is complicated by the vastly different morphology of these structures. Histologically, however, the bulbo-vestibular gland is very similar to the smallest lobe (Cowper's 3) of the male. The less convoluted paired ducts of the female gland enter the urogenital sinus in approximately the same position as the paired common ducts of the male gland, that is, on the ventro-lateral aspect. The counterparts of Cowper's 1 and 2 do not develop in the female, a condition which probably accounts for the relatively small size of the gland in comparison with the Cowper's complex of the male.

IV. EXPERIMENTAL TREATMENTS

ANDROGENS

Moore ('41a, '41b) has demonstrated that the prostate gland of the male opossum, from the time of its first appearance to the latest stages studied, can be precociously stimulated by administered androgens. Moreover, similar treatments of young female opossums induced prostate gland formation (which never occurs in normal female development) and stimulated them to a degree exceeding that of normal untreated males of the same age. In view of these findings, and the fact that the anlagen of Cowper's glands appear in both sexes at the

same age in normal development but undergo dichotomous differentiation, the desirability of studying the effects of androgens upon Cowper's glands in the male and female opossum becomes evident. Would administered androgens hasten the development of Cowper's gland in the male, and could they effect a male type of development in the homologue of the female?

The androgenic substances were administered either as daily percutaneous applications of lanolin base ointments containing free testosterone or testosterone propionate (2 mg. per gram), or as daily (or in some cases on alternate days) subcutaneous injections of testosterone propionate in sesame oil. The dosages actually received via the percutaneous method increased as the body surface of the developing young increased, and are for obvious reasons not accurately estimable. That sex hormones are, however, effectively absorbed through the skin has been demonstrated in the opossum and other laboratory mammals (Moore, '41a; Moore, Lamar, and Beck, '38; Scott, '40). The subcutaneous injections prior to day 70 were administered in 0.25 mg. amounts, in 0.05 cc. of oil; after day 70 the amounts varied from 0.5 mg. to 5.0 mg. Minor variations in procedure will be explained in the appropriate places in the text. Forty-one androgen-treated animals were used—17 immature males (16–150 days old); 23 immature female (16–150 days old); 1 adult female (anestral).

A. Effects on Cowper's glands in the male

For purposes of comparison it appears advantageous to discuss the androgenic effects in male opossums under three headings as follows: (1) treatments begun prior to the appearance of the anlagen; (2) treatments begun after the appearance of the anlagen but prior to early morphological differentiation—that is, into the three lobes (Cowper's 1, 2, and 3); and (3) treatments initiated after morphological differentiation—during later pouch life and subsequent to 100 days.

Effects of treatments initiated before day 5. Four pouch-young male opossums treated with androgens prior to the embryological origin of Cowper's glands anlagen (5th day) were sacrificed at ages from 16–36 days. This covers the period of normal differentiation into the three paired lobes, Cowper's 1, 2, and 3.

Cowper's gland anlagen of opossum 96A, treated from day 3–15 and sacrificed on day 16, was in an advanced condition as compared with a 16-day normal male (opossum 33A). All of the structures in the region of the anlagen, including the urogenital sinus and the corpus

cavernosum, were markedly enlarged. Cowper's anlagen were in a stage of development equivalent to those of the 24-day normal male, opossum 10B. Some slight indication of dorsal branching was apparent in the region of the mesenchymal areas destined to become the connective tissue and muscle capsule of Cowper's 1 and 2. Proximally the otherwise solid cords had small lumina which did not persist to the urogenital sinus epithelium. In untreated normal stages the appearance of cavities in the anlagen of Cowper's gland does not occur until after day 24. Volumetric measurements (table 1) reveal the fact that the total tissue volume of the 16-day androgen-stimulated anlagen is approximately the same as that of the untreated 24-day stage, and 2.6 times that of the normal 16-day stage.

In opossum 47A, treated from day 3-24 and sacrificed on day 24, no evidence of branching was apparent. The heavy cord-like anlagen were hollowed throughout most of their length and extended dorso-laterally, at an approximate 45° angle, from the lateral wall of the sinus epithelium. Cellular hyperplasia was evident throughout the unbranched, hollowed cords. The lumen was lined by polyhedral cells (with extremely light cytoplasm) which resembled the "embryonic" epithelium lining the urogenital sinus canal. In some sections sloughed off cells appeared in the lumen, but these were few in number. The cords opened by relatively large orifices into the ventral part of the urogenital sinus canal, a departure from the condition in untreated males where patent ducts occurred for the first time in the 52-day stage. Volumetric measurements revealed a three-fold and a two-fold increase in the size of the anlagen as compared with the normal 24-day and 32-day stages, respectively.

Male 101A, treated from day 3-15 with ointment, containing testosterone, and from day 16-29 with injected testosterone propionate, was sacrificed on day 30. The anlagen (fig. 12) have become very thick canalized cords of cells. The epithelium is many layers thick, is glandular, and forms folds which project into the central lumen. Stimulation of the muscle and connective tissue representing the capsules of Cowper's 1 and 2 is very evident, but no dorsal branchings of the Cowper's gland anlagen have occurred. The only indication of an attempted differentiation into lobes is a dorsal extension of the anlagen toward the pear-shaped mesenchyme of the capsule of Cowper's 1. In contrast to this condition the epithelium of the 32-day untreated male is undifferentiated, the branching of the main cord into the lobes of Cowper's 1, 2, and 3 is already under way, and the ducts, while hollowed in their proximal parts, are as yet not patent into the sinus canal.

A section of the urogenital sinus portraying the condition of the anlagen in the treated male is shown in figure 12, which is photographed at the same magnification as is the section of the untreated 32-day male in figure 9.

Cowper's gland of opossum 52, treated from day 3–35 percutaneously, and sacrificed at 36 days, appeared slightly less modified than the preceding. The outgrowths, while large and canalized throughout, were not as greatly stimulated as those of the 30-day androgen-treated male (opossum 101A). In cross section, morphological differentiation is manifest to some degree in the development of small clusters of cells at the base of Cowper's 1 mesenchymal capsule. The pear-shaped and oblong stromal capsules of Cowper's 1 and 2, respectively, are intermediate in size between the 30-day treated and the 32-day normal male specimens. The sinus canal lining is likewise less stimulated and more nearly normal. These apparent discrepancies in the results of androgenic treatments are easily accounted for by considering the difference in dosages and lengths of treatment. Thus, opossum 52, the animal under consideration, received cutaneous applications of testosterone for 35 days, whereas the 30-day-old animal, opossum 101A, received the same treatment for 13 days (days 3–15) followed by daily injections of testosterone propionate from day 16–29. Testosterone propionate, given subcutaneously, has a much greater stimulating effect on Cowper's gland anlagen and neighboring structures than do percutaneously administered androgens. The difference in effect is obviously a question of the availability of hormone.

Effects of treatments initiated before morphogenesis. Serial sections of eight specimens, sacrificed from day 22 to day 39, were available for determining the effects of androgen administration initiated during the crucial period from the 10th to the 16th day — that is, just prior to branchings of the primordia of Cowper's 1, 2, and 3 from the main cord. Only four cases will be considered in detail.

In opossum 63A — treated from day 10–21 with testosterone propionate injections, and sacrificed on day 22 — the condition of the anlagen was essentially the same as that described for opossum 47A, the 24-day stage, in which treatment was initiated on day 3. The total volume of Cowper's tissue was four times greater than that of the normal 24-day-old specimen and somewhat greater than that present in opossum 47A (table 1). Only meager evidence of morphogenesis was apparent in the formation of a small, solid bud of cells originating from the main cord at the base of the pear-shaped mesenchymal capsule of Cow-

per's 1. Mitotic figures were abundantly scattered throughout the anlagen.

In another specimen, opossum 38B, treated from day 15-25 with cutaneously administered androgens, and sacrificed on day 25, the stimulating effect was less marked. The cords show a slight enlargement and are tubulate proximally, but do not open into the sinus canal. The anterior-most parts are branched and the formation of a distinct Cowper's 3 is evidenced. There is decidedly less retardation of morphogenesis in this animal than in any of the preceding ones. Moore ('41a) reported other reproductive parts in this animal as being little stimulated, when compared with other androgen-treated specimens.

The 31-day Cowper's gland of opossum 64A, treated on alternate days from day 10-30 with injections of testosterone propionate was greatly stimulated. The description given of the condition in opossum 101A is equally applicable to this specimen. There appears to be a more highly convoluted, stratified epithelium lining the lumen of the dorso-lateral cords and an abundance of mitotic figures. Here, as in opossum 101A, the canalized anlagen open into the sinus canal by way of an orifice measuring approximately 0.08 mm. in diameter as compared with a diameter of less than 0.01 mm. in the anlagen of the untreated 52-day male. An increase in the width of the cords appears to have progressed at the expense of the dorso-ventral lengthening and branching. Comparisons should again be made of figure 12 (opossum 101A) with figure 9 (opossum 34B) for an understanding of this condition.

The last specimen of this series, opossum 65A, was treated from day 10-31 with injections of testosterone propionate. Treatments were suspended subsequent to day 31, and the animal was preserved for study on day 39. The anlagen of Cowper's glands were heavy and broadly cavitate cords. Branching was greatly reduced, but less so than in continuously treated specimens. Cellular debris was also less apparent than in continuously treated animals. The maximal diameter of the anlagen was 0.216 mm. Perhaps as a result of discontinued treatment in the last 8 days of life the urogenital sinus was apparently normal. In the majority of cases just considered the urogenital sinus in the region of Cowper's gland was metaplastic and showed signs of cornification—a reaction previously reported by Burns ('39a, '39d). As a rule the distal part of the sagittal urethral plate in the shaft of the phallus was not only cornified but also hollowed out. In normal development the distal part of the sagittal urethral plate does not hollow out until a much later stage.

A summary of the effects of androgens in the development of Cowper's gland anlagen during early growth and morphogenesis includes a number of interesting observations. These are essentially the same whether treatments have been initiated prior to the first appearance (5 days) of the anlagen, or prior to the first stages of morphogenesis — normally occurring in the untreated normal 24-day male. For convenience the effects may be listed as follows: (1) a general stimulating effect resulting in an increased rate of growth and in advanced epithelial differentiation and canalization of the normally solid cords; (2) a retardation of the normal development processes with consequent interruption of morphogenesis; (3) the occurrence of loose cells, mainly leukocytes, in the hollowed portions of the stimulated cords — a condition which occurs also in the juvenile stages of 100–150 day normal males; and (4) a precocious patent contact with the urogenital sinus canal.

In addition to these effects on Cowper's gland, there is marked stimulation of the erectile bodies and the epithelium of the sinus canal. The latter is hyperplastic, metaplastic, and cornified in the region of Cowper's gland, as is also the precociously-hollowed, distal portion of the urethral plate.

Effects of treatments subsequent to morphogenesis. In this portion of the experimental study, four males, aged 83–150 days, have been treated with injections of testosterone propionate. Two animals received daily injections of 0.5 mg. beginning on day 70. One was preserved after thirteen injections on day 83, the other, after thirty injections, on day 100. The two remaining animals of this group were injected daily with 2.0 mg. for 13 days and sacrificed when approximately 5 months of age.

Androgenic treatments initiated during later pouch stages and in juvenile male animals have resulted in a pronounced stimulation of all parts of Cowper's complex. Comparisons with identically-aged untreated normal males reveals marked increases in size and degree of histological differentiation in all three lobes (Cowper's 1, 2, and 3), and in all parts of the duct system. The majority of tubules, even the most peripheral ones, are hollowed out. Many of the lumina enclose a homogeneous staining, translucent material — probably a secretion product. The ducts are large, convoluted, and partitioned into a number of longitudinal divisions by transverse, epithelial-lined septa. The cells of the ducts are tall columnar and are glandular in appearance. In addition to the response of the epithelial elements, the muscle tissue is greatly hypertrophied. Mitotic figures are much more abundant

than in normal animals of the same age and no sign of cellular debris has been observed. In the 100-day-old animal, opossum 98, treated for 30 days with a total of 15,000 gamma of androgen, the glandular elements were almost the equivalent of the adult condition. Cowper's 1, the largest lobe, had large lumina lined with low cuboidal epithelium, probably the result of distention produced by the enclosed colloid. The lumina were of variable dimensions, but the largest were approximately ten times greater in diameter than that of the largest lumina in the same lobe of a normal 150-day gland. Cowper's 2 was equally stimulated although the tubular distention was less apparent and the cells were medium columnar. Least stimulated of the three lobes was Cowper's 3, but even here the development was in advance of the normal 150-day condition. In general a picture of intense stimulation with marked secretory activity is manifest. Muscular and erectile elements are hypertrophied to surprising proportions in all cases.

TABLE 3

Greatest diameters of Cowper's 1 and 2 and their ducts in 150-day normal and 100- and 150-day treated opossums.¹

SERIAL NUMBER	AGE AT DEATH	TREATMENT ²	COWPER'S 1		COWPER'S 2	
			Gland	Duct	Gland	Duct
	(days)					
177	150	Normal control	2.16	0.49	2.07	0.36
98	100	0.5 mg. TPI — 30 days	2.61	0.90	2.61	0.72
172	150	0.5 mg. TPI — 13 days	3.96	0.99	3.15	0.81
168	150	40 R.U. Gd — 14 days	3.70	0.63	2.96	0.54
169	150	40 R.U. Gd — 14 days	3.78	0.72	3.06	0.54

¹ Ocular micrometer measurements in millimeters.

² TPI, testosterone propionate injections; Gd, gonadin; R. U., rat unit.

The injection of larger doses (2.0 mg.) in 5-month males over a 13-day period produced even greater stimulation than in the 100-day gland. The effect is best demonstrated by comparisons of figures 18 and 19 with figures 14 and 15, which show small areas of the normal and treated 150-day Cowper's glands, respectively. Ocular micrometer measurements of the 100- and 150-day treated glands and ducts, and identical measurements for the corresponding 150-day normal structures are shown in table 3.

Thus, it may be concluded that androgenic treatments initiated subsequent to the attainment of the definitive morphological state of Cowper's gland induce precocious development within all three lobes which simulates a more advanced condition in normal development.

B. Effects on Bartholin's glands in the female

The stimulating effect of androgens on the female homologue of Cowper's gland in the opossum has been mentioned in the work of Moore ('39, '41a) and Burns ('39a, '39c). However, no specific details were reported in either publication. The following results deal with the findings obtained in androgen-treated Bartholin's glands, and have, for purposes of convenience, been divided into three age categories: (1) the first 100 days; (2) the 150th day; and (3) the adult.

Effects of treatments up to 100 days. Twenty-one females, ranging in age from 16–95 days at the time of preservation, received varying treatments with androgens. The mode of administration was the same as that enumerated previously for the treated males. Up to and including 39 days, there were available comparable androgen-treated sets of male and female specimens. In these females, aged 16–39 days, the effects of androgens on the development of Bartholin's glands were entirely comparable to the effects already described for Cowper's glands of the male. In a normal 44-day-old female the solid epithelial parts of the gland are situated just beneath the smooth musculature on the ventral aspect of the urogenital sinus. In the 39-day female, opossum 65B (injected with testosterone propionate from day 10–31), the gland anlagen are in a condition equivalent to that described for the 39-day androgen-treated male. The only difference is in the degree of stimulation—the female gland responding to a slightly lesser extent. Relative volumes (table 2) of the amount of tissue present in representative stages of pairs of littermate male and female animals from day 16–31, indicate an almost equivalent response by both sexes. A comparison, however, of treated and normal gland volume measurements of individuals of the same sex indicate a much greater deviation from the normal in androgen-treated females than in androgen-treated males. A histological difference, of doubtful significance, was the absence in Bartholin's gland anlagen of sloughed-off cells within the hollowed cords. In littermate males receiving the same treatment the presence of cellular debris within the hollowed cords may be due to the greater degree of hyperplasia which occurs in the male gland.

In opossum 36, a 41-day female (treated from day 16–40 with testosterone ointment), the gland appeared to the lateral sides of the urogenital sinus as tubulate cords. The high epithelium lining the lumina continued to within a short distance of the openings into the urogenital canal. The openings into the canal were by way of papillae which penetrated the mucosa obliquely and coursed for a short distance

posteriorly within the sinus lumen. This mode of entrance into the canal is characteristic of older males, although a similar condition was noted in the 67-day normal female. In normal development the first sign of duct patency was also observed at 67 days.

The typical early male effect of androgenic stimulation, with attendant retardation of terminal branching, was manifest in opossum 42, a 51-day female treated with testosterone ointment from day 16-51. The epithelium was greatly stimulated and the cords were tubulate throughout. The ducts were highly convoluted and opened by way of papillae into the sinus canal. The lining epithelium of the ducts was high columnar and folded. The terminal branching of the treated specimen is minimal as compared with the degree of branching in the normal 44-day animal. The lumina of the distal parts were distended and the lining cells were cuboidal to low columnar. Small areas of colloid-like material were present in a few lumina. No evidence of cellular debris was apparent in any of the parts. Most of the stimulated glandular parts were imbedded in the body of the corpus spongiosum. The muscular and connective tissue, and erectile body elements were greatly stimulated; the corpus cavernosum clitoridis of this specimen had a dorso-ventral dimension two to two and one-half times greater than that of a normal 67-day female.

The effects of long periods of androgen treatment on Bartholin's glands were studied in sections of opossum 117, sacrificed on day 95 after 91 days of hormone treatment (3-15 testosterone, ointment, 16-93 testosterone propionate injections on alternate days). Contrary to the effects of this hormone on induced female prostates, the effect on Bartholin's gland was unimpressive. The lumina were large and some cellular desquamation was evident, whereas the glandular parts were not as well developed as in a normal female of 83 days. The epithelium varied from cuboidal to columnar. Peripheral expansion was limited, and the glandular parts occupied a position near the lateral aspects of the sinus. The ducts entered the sinus canal in the usual manner — that is, without the formation of papillae. The urogenital sinus epithelium was not as hyperplastic as in the younger stages and only slightly cornified.

Thus, prolonged androgenic treatments have not only failed to exert an acceleration of normal growth processes in the developing female homologue of Cowper's gland, but have actually disturbed the normal pattern of development — as judged by comparisons with normal stages. A similar condition has already been described in the early androgenic treatments of Cowper's gland in the male. The bearing of these re-

sponses on the general problem of sex hormone influences during sexual differentiation is of the greatest interest and will be more fully discussed in another portion of this paper.

Interrupted androgen injections, that is, treatments given during early pouch life and then discontinued for a short and a long interval prior to sacrifice, provided instructive conditions for comparison with continuous treatments. Two animals treated in this manner will be considered in detail.

Opossum 66, was treated daily from day 10-23 and on alternate days from day 24-31 with subcutaneous injections of testosterone propionate. After treatment was discontinued on day 31, the animal was sacrificed on day 62. Microscopically a mixed effect was observed in the condition of Bartholin's gland. The stimulating effects described in earlier stages were still apparent in the form of luminal areas lined by columnar epithelium and in patent ducts opening into the sinus canal. On the other hand, the cessation of androgenic administration appears also to have facilitated the re-establishment of normal growth processes. This is concluded from the presence of terminal proliferations of solid cords of cells which resemble the branchings of the cords in the 44- and 67-day normal females. Sloughed-off cells occur in certain lumina, perhaps as a result of hormone removal. There has also been some regression in the hypertrophic erectile tissue and surrounding muscle.

Opossum 118 received cutaneous applications of testosterone from day 3-15 and injections of testosterone propionate from day 16-44. Subsequent to day 44 no further treatments were administered and the animal was preserved on day 95. Histological examination of Bartholin's gland revealed a more extensively branched and a more diffuse condition than that which occurred in the gland of opossum 117 (treated continuously from day 3-93). There was a marked tendency toward a return to the normal female condition. In the anterior region normal growth processes seemed to have been re-established in the form of a number of non-tubulate proliferations, and more posteriorly the bilaterally situated parts extended toward the mid-ventral line, and the tubules of each side came to lie in contiguous areas. Cessation of treatment appears, as in the preceding case, to have facilitated a return in the direction of normal development. A comparison of figure 17, a cross section of the 95-day treated gland, with figure 13, a cross section of a normal 100-day gland shows certain differences which still exist.

Effects of treatments at 150 days. The reaction of the juvenile gland to androgens is entirely different from that of the younger stages. Two females, opossums 174 and 175, were treated with daily subcutaneous

injections of 2.0 mg. of testosterone propionate for 13 days. The effect is essentially the same in both, and the results will be discussed together. Comparisons with the untreated Bartholin's gland of 150 days reveal a number of important differences. The treated glands are larger in size, and, histologically, are better developed. The antero-posterior dimension of one gland was 4.06 mm. and that of the other 4.93 mm., as compared with 3.40 mm. and 3.53 mm. in the two untreated controls. In addition to the antero-posterior increase in size, the medio-lateral dimensions are approximately twice as great as in the normal gland. In the anterior two-thirds of the gland the epithelium is tall columnar, and the tubules are closely packed with but little connective tissue between them. The epithelium of the posterior third of the gland is medium to low columnar, the tubules are further apart, and the inter-tubular tissue is considerably increased in amount. All portions of the gland are hollowed out and the tubules are larger than are those of equal-aged normal controls. Conspicuous accessory lobules of glandular tissue occur on one side of the gland in opossum 175. They extend laterally from the ventral aspect of the main body of the gland and join with it more anteriorly. These extensions are shown in figure 21. Unlike the condition in normal glands of 150 days, there is practically no sloughing of cells into the lumina (compare fig. 20 and 21). The short ducts leave the medial aspect of the posterior part of the gland, penetrate the urethral mucosa, and open into the ventro-lateral walls of the sinus by wide apertures. The glandular epithelium of the ducts is continued to within a very short distance of the stimulated, but not cornified, sinus mucosa.

Androgen treatment in the adult. The effects of androgens were studied in sections of Bartholin's gland removed from an anestrus adult female subsequent to twenty daily injections of 5 mg. amounts of testosterone propionate. The tubules were large, distended and closely packed, with very little intervening connective tissue. The cells were tall columnar with large, deeply-staining nuclei. Seen within the lumina of the peripheral tubules was an evenly-staining colloid, similar to that found in Cowper's glands of the adult male. Medially the glandular elements appeared less stimulated, but were, nonetheless, more in accord with the male appearance than with that of the untreated female. The accessory lobules, mentioned in normal development, were tremendously stimulated by androgens and extended laterally and antero-posteriorly for some distance. In gross dimensions this gland was larger than any of the normal or other treated adult glands studied.

In résumé, androgenic effects on Bartholin's gland in the early pouch-young female opossum are essentially the same as those produced in Cowper's gland of early pouch-young males — that is, pronounced stimulation of the processes of canalization and epithelial differentiation, but a decided interference with the normal mode of development displayed by the untreated gland. The latter phase becomes even more apparent in the case of prolonged treatment from day 3 to day 95. Shorter treatments which are subsequently discontinued result in a re-establishment of the developmental trends characteristic of the normal gland. Unfortunately there are no specimens available which have been kept for a sufficiently long period of time to determine whether or not complete recovery can be achieved. In stages subsequent to 100 days the effect has been one of stimulation which simulates the response of the epithelial elements of the gland of similarly aged males. The response is, however, effected without a distortion of the general structure characteristic of the normal female gland. Thus, except for the pronounced stimulation of the accessory lobules, androgens do not effect a morphological shift of Bartholin's gland in the direction of the male homologue.

ESTROGENS

Effects of estrogenic substances on the reproductive tract of the opossum, in males and females and in early and later developmental stages, have been presented in detail by Moore ('41a). In males the effect of early estrogenic hormone administration on the accessory sex gland most studied — namely, the prostate — has been one of complete suppression. This effect, which Burns ('42a) claims is complete and permanent, is the result of a central rather than a peripheral proliferation of the urogenital sinus epithelium (Moore, '41a). In later stages estrogens were found to convert the well developed prostatic outgrowths into hyperplastic and metaplastic cell masses. In estrogen treated females, of course, no prostatic tissue is to be expected, and none occurs. In view of the damaging effect of estrogenic substances on prostate development, it was of interest to study the effects of these substances on the development of Cowper's gland and its female homologue.

The estrogens were administered to the pouch-young animals mainly in the form of ointments (containing 0.625 mg. of estradiol per ounce) applied on the skin. As in the case of similarly administered androgen ointments, the dosages are not accurately known. It is, however, estimated that daily doses of approximately 1 gamma of estradiol were applied to young pouch-animals and not more than 6 gamma to older

pouch-animals. In a few of the earlier stages and in all of the later stages subcutaneous injections of oil solutions containing either Progynon B (the benzoic acid ester of the dihydro follicular hormone, estradiol) or theelin were used. Despite the extremely lethal nature of such treatments on pouch-young animals (attributed by Burns, '42a, to urinary retention resulting from the occlusion of the excretory passageways as a result of extreme hyperplasia and cornification) a number of specimens were obtained for detailed studies of Cowper's gland in the male and Bartholin's gland in the female. Comparisons have been made primarily of the histological appearance of the glands in the treated animals with that of the glands in untreated controls of the same age, and with that of androgen-treated structures.

A. Effects on Cowper's glands in the male

The mode of presentation of the results will follow after the pattern outlined in the discussion of the androgen effects, as follows: (1) treatments begun at 5 days and prior to early morphological differentiation; (2) treatments begun during early morphological differentiation; and (3) treatments begun after morphological differentiation — during later pouch life and subsequent to 100 days.

Effects of treatments initiated on day 5 and prior to morphological differentiation. Six pouch-young males, aged 12–31 days when sacrificed, were treated for different consecutive periods of time, from day 5–30, with estradiol in ointment.

Three specimens, opossums 97, 49A and 55A, in which treatment was begun on day 5 and terminated on day 11, 16, and 22, respectively, exhibited essentially the same condition. In normal 16-day specimens the anlagen are narrow, solid cords extending dorsally from the sinus epithelium and are beginning to show evidences of terminal branching (fig. 4). In opossums 49A and 55A the Cowper's anlagen are short, thick, hollowed cords lined by stratified epithelium and in contact with solid hyperplastic lateral extensions of the distorted lower urogenital sinus. The epithelium of the urogenital sinus in the region of the gland anlagen is hyperplastic, metaplastic, and completely cornified. However, no cornification is evidenced in the cords of Cowper's gland primordia.

These findings introduce two important points, as follows: (1) estrogens do not suppress the formation of Cowper's gland anlagen, and (2) estrogens fail to induce cornification in these anlagen. The first of these conditions differs from the situation found in a study of the estrogenic effects on the prostate, but may perhaps be due to the fact

that Cowper's gland anlagen appear at a much earlier stage in development than does the prostate. Thus, the anlagen may have been able to establish themselves before the action of the estrogens became effective. On the other hand, until earlier treatments are initiated, no conclusive statement can be advanced. The point regarding the absence of a cornifying reaction in Cowper's gland anlagen and its significance, in terms of recent reports (Burns, '42a), will be discussed later.

In opossum 59, treated with daily injections of 125 I.U. of Progynon B from day 10-16 and sacrificed on day 17, the sinus was considerably less cornified. Cowper's anlagen were correspondingly less hyperplastic, although completely tubulate, and gave evidence of slight convolutions at the terminal end; this was more like the condition in the androgen-treated males. In addition, there was a lack of the lateral, hyperplastic extensions from the urogenital sinus, so that the anlagen originated from the ventral aspect of the sinus epithelium. The corpus cavernosum was well developed and appeared stimulated.

The oldest members of this series, opossums 109 and 39A, were treated with estrogen ointment from day 6-29 and 17-30, respectively; the former was sacrificed on day 30, and the latter on day 31. Cowper's gland anlagen in normal males of 31-32 days is recognizable as a divided structure in its terminal extent where distinct branchings, which represent Cowper's 1 and 2, have already appeared. The proximal ducts are slightly canalized, but the remaining parts are solid epithelial structures. In the 30-day estrogen-treated male Cowper's anlagen extend to each side of the extremely hyperplastic and cornified urogenital sinus as single, hollowed, heavy cords. In anterior cross sections each cord appears as an unbranched tubule lined by 5-6 layers of stratified epithelium, and situated on the latero-ventral aspect of the smooth musculature of the sinus. Unlike the anlagen of androgen-treated males of the same age, the cords show no signs of folding. At the point of sinus origin they are attached to lateral, hyperplastic extensions of the urogenital sinus, and appear to be patent, by way of very narrow channels, into the distorted lower sinus canal. The latter observation was made uncertain as a result of the thickened, metaplastic epithelial wall of the sinus.

In the 31-day estrogen-treated male the condition of the gland anlagen and the urogenital sinus is essentially the same as that described for opossum 109. Two exceptions, however, are apparent: one is the slightly coiled condition of the tubulate cords at their anterior ends, and the other is the presence of a dense, homogeneous colloid in portions of these cords. The glandular epithelium and the anterior convolution

of the cords resemble the condition in opossum 101A, the androgen-treated 30-day-old male. This resemblance can be seen in a comparison of figures 12 and 11, similarly magnified photomicrographs of the androgen- and estrogen-treated glands. The reason for the anterior convolution of the anlagen of opossum 39A as compared with the simple uncoiled condition of the anlagen in opossum 109 is probably due to the difference in the time at which treatments were initiated. It should be recalled that the 16-day normal gland is already slightly branched at its terminal end.

Effects of treatments begun during early morphological differentiation. Cowper's glands of two animals, injected with daily doses of 25 I.U. of theelin from day 32-43 and sacrificed on day 43, were studied in serial section. The normal condition at 32 days, which corresponds to the period at which treatments were begun, has been reviewed above. The normal 44-day gland, corresponding to the time at which treatment was discontinued, is in an advanced stage of development. The three lobes, Cowper's 1, 2, and 3, are well defined and exhibit marked branching within their respective capsules; however, all of the local branchings are solid epithelial cords, except where in contact with the ducts. These ducts are hollowed, and the lining epithelium is acquiring a glandular appearance in the distal parts. In contrast to this condition of the normal 44-day gland, the estrogen-treated 43-day gland is entirely tubulate in all of its parts. There has been, however, no morphological advance in the condition of the lobes of Cowper's 1, 2, and 3 beyond that described for the 32-day stage.

It may be concluded that estrogens administered at the time of Cowper's gland origin have not interfered with its appearance, but, as in the case of androgens, have stimulated the epithelial elements and have interfered with the normal morphological development. Similarly, treatments initiated during early stages in the morphological development of Cowper's gland have effectively arrested such development and have instead stimulated histological differentiation. The degree of histological stimulation has not been as great as that produced by androgens.

Effect of treatments begun after morphological differentiation. Four male animals representing older stages in development were available for a study of the effect of estrogens on Cowper's gland. All of the specimens received daily injections of Progynon B and were sacrificed on the day following the last treatment.

Opossum 93 received 20 I.U. daily from day 70-82 and was preserved on day 83. All parts of Cowper's gland were greatly stimulated in comparison with the normal 83-day stage. However, there is a marked

sloughing of cells into the lumina of the various lobes — a condition which is most striking in Cowper's 1 where the cast-off cells of the stratified and hyperplastic columnar epithelium are mixed with numerous leukocytes. The latter have infiltrated all of the lobes and ducts, and are thoroughly mixed with the cellular debris. This reaction appears to be the same as that reported by Wells ('36), in which an extensive leukocyte invasion accompanied the atypical estrogen stimulation of Cowper's gland in the male ground-squirrel. The epithelium of Cowper's 2 and 3 is affected in much the same way, and the connective tissue stroma of all three lobes shows marked hyperplasia. The common ducts open into the urogenital sinus canal after traversing the 10–12 layers of stratified hyperplastic and metaplastic epithelium with which the canal is lined.

Opossum 95 was sacrificed on day 88 after having received daily injections of 20 I.U. of Progynon B for 18 days. The most interesting feature of the prolonged period of treatment was the hyalinization of the epithelium of the common ducts and the duct of Cowper's 1. At their points of origin from the sinus epithelium the lining of the ducts is identical in structure with the hyperplastic epithelium of the sinus mucosa. In the duct of Cowper's 1 there is a gradual transition from the basal layer of deeply-staining columnar cells to the central layer of hyaline, polyhedral cells which line the lumen. Aside from this added effect, the other changes do not differ materially from those described in the preceding case. In spite of the intense stimulating effect of estrogens on the glandular epithelium (which in some areas is so tall as to actually occlude the lumina), and connective tissue of the three lobes of Cowper's gland, the general effect is decidedly atypical and unlike the effect produced by androgens. In similar-aged animals androgenic stimulation results in precocious development simulating the adult condition; there is neither desquamation of epithelial elements nor infiltration of large numbers of leukocytes.

In two juvenile males (opossums 165 and 166) of approximately 150 days, the daily injection of 100 I.U. of Progynon B for 10 and 11 days failed to materially affect the epithelial elements of Cowper's gland. The main effect seems to have been a slight stimulation of the connective tissue elements, which is most conspicuous in Cowper's 3. Some of the lumina contain small amounts of cellular debris which are negligible when compared to the amounts present in opossums 93 and 95.

The nature of the effect of estrogens on Cowper's gland in 70-day-old and in 150-day-old animals is unquestionably different. The factor

responsible for this difference is probably the male hormone secretion of the 150-day gonad, but which is not yet being secreted — or if at all, in insignificant amount — by the 70–80 day gonad (experimental results reported in this paper as well as those reported by other investigators in this laboratory indicate that the opossum gonad probably does not become functional until approximately 100 days, if then). Thus, the 70–80 day male is, to all purposes, a physiological castrate in which small amounts of estrogen are capable of stimulating the epithelial, muscular, and connective tissue elements of Cowper's gland in much the same way as this substance is known to affect the male accessory sex glands of other mammals (Wells, '35, '36; Weller, Overholser, and Nelson, '36). The absence of such effects in the 150-day male gland may have been due to the small amounts of estrogen used, since it is known that larger amounts of estrogen are required to induce the above changes in normal animals than in castrates (Allen, Hisaw, and Gardner, '39).

B. Effects on Bartholin's glands in the female

The effects of estrogens on Bartholin's glands of the female opossum were studied in fourteen pouch-young stages ranging in age from 17–79 days, in two juveniles (150 days), and in one anestrus adult. Some of these are listed in table 4 together with several estrogen-treated male specimens. The pouch-young were treated with cutaneous applications of estrogen ointment, the juveniles and adult with injections of Progynon B.

Effects of treatments in pouch-young. The effect of estrogens on Bartholin's glands of pouch-young stages are entirely comparable to those enumerated in detail for similar-aged and similarly treated males. The differences between male and female responses, when they exist, are quantitative and not qualitative; thus, the urogenital sinus of the estrogen-treated females is more greatly distorted and shows greater hyperplasia and metaplasia than does the estrogen-treated male sinus.

Only one case in this group need be considered in detail — namely, opossum 119 which was treated from day 6–45 at which time treatments were discontinued until sacrifice on day 79. The most conspicuous difference between Bartholin's gland of this animal and those of the continuously treated glands of animals sacrificed on day 45, was in the presence of a number of solid buds which appeared to have proliferated from the large hollowed portions resulting from the earlier treatment. The presence of the solid buds, appearing as solid cell clusters in cross section, is a condition which seems to indicate a re-

sumption of normal growth processes. The solid, newly proliferated areas are composed of cells which are histologically like those of normally developing glands, and which contain many mitotic figures. The glandular parts are enclosed in concentric layers of connective tissue and some striated muscle fibers. Hyperplastic and metaplastic changes are in subsidence, the latter almost entirely absent. A persistent effect of the early estrogenic treatment remains in the form of a double

TABLE 4

Sample data of estrogen-treated male and female opossums.

SERIAL NUMBER	AGE AT DEATH	TREATMENT ¹	SNOUT- RUMP	HEAD LENGTH	WEIGHT
Males					
	(days)	(days)	(mm.)	(mm.)	(gm.)
49A	17	5-16 EsO	32.5	11.0	1.27
55A	23	5-22 EsO	33.5	11.8	1.36
109	30	6-29 EsO	49.7	16.7	3.88
39A	31	17-30 EsO	46.0	15.4	3.03
37B	43	32-43 Th		...	
93	83	70-82 PB	123.0	41.0	56.00
95	88	70-87 PB		...	
165	150	140-149PB		...	260.00
Females					
49B	17	5-16 EsO	33.5	11.0	1.50
55B	23	5-22 EsO	33.0	11.8	1.35
108	30	6-29 EsO	48.6	15.8	3.81
106	35	6-34 EsO	69.0	19.1	7.09
110	43	6-42 EsO	67.7	21.3	7.58
		6-45 EsO			
119	79	46-79 no tr.	123.0	43.2	59.00
170	150	138-149PB		...	290.00

¹ EsO, estrogenic ointment; Th, theelin injection; PB, Progynon B injection; no tr., no treatment.

set of ducts on one side of the sinus, one of which appears to be an aberrant offshoot from the main cord. In the earlier stages, as a result of the imperfect relations of the urogenital sinus, the ducts of Bartholin's glands were found to open very close to the external orifice of the urogenital sinus. In this specimen the duct openings were no longer located near the orifice, but opened into a more proximal portion of the sinus canal. It would appear that the deleterious effect of estrogens on the normal development of Bartholin's glands can be remedied to

some extent following the cessation of treatment. This situation is the same as that discussed in androgen-treated females.

Effects of treatment in the 150-day stage. Two 150-day-old females received daily injections of 100 I.U. of Progynon B for 12 days and were sacrificed on the day following the last treatment. The histological picture of Bartholin's gland was essentially similar to the condition observed in the same-aged normal gland (fig. 20). Cell height varied from cuboidal in the peripheral tubules to medium columnar in the central tubules. Sloughed cells mixed with leukocytes were found in practically every tubule, just as in the normal gland. In only one particular does the treated gland deviate from the normal and that is in the condition of the ducts. In the normal 150-day gland the stratified epithelium of the urogenital-sinus mucosa invades the duct to a distance of 0.09 mm., whereas in the estrogen-treated gland the stimulated epithelium of the sinus penetrates the ducts to a distance of 1.62 mm. There is, in the latter, an abrupt transition from the hyaline, stratified, squamous epithelium to the folded, glandular epithelium which lines the remainder of the duct. In gross size the treated glands were larger. They measured 7.5 mm. from one lateral border to the other across the central part of the gland in the region of mergence. The same measurement taken of the normal 150-day gland was 4.0 mm. The histological condition of both the normal and estrogen-treated 150-day glands is similar to that which was described in the 83-88 day estrogen-treated Cowper's glands of the male.

Effects of treatment in an anestrus female. To determine the effects of estrogenic treatment in an anestrus adult female, a single specimen was injected daily with 100 I.U. of Progynon B for a period of 12 days. The urethral mucosa was stimulated to tremendous proportions as was also the size of the urethral lumen. Bartholin's gland retained the general size characteristic of the normal anestrus gland, but the glandular tissue was drastically reduced and the connective tissue relatively increased in amount. The majority of tubules contained an abundance of cellular debris and lacked any trace of colloid normally present in untreated animals. Figure 24 is a highly magnified area of the gland showing the nature of the tubular epithelium and the connective tissue stroma. This may be compared with figure 23 which shows a similar area in a normal anestrus adult. The interesting feature of this result is the difference in histology exhibited by Bartholin's gland of the treated anestrus adult opossum as compared with that of the untreated adult. Theoretically it might be expected that the condition would simulate that which prevails in the breeding

opossum, but comparisons of figure 24, showing the treated gland, with figure 22, showing the untreated breeding gland, soon dispel any notion regarding such identity. In the section on gonadotropic effects this question will be elaborated upon more fully, and a possible explanation will be advanced.

GONADOTROPINS

It has been reported that the prostate gland of the 70-day-old male opossum exhibits the earliest unquestioned response to hormones produced by the animal's own gonad under the influence of injected equine gonadotropins (Moore and Morgan, '43). At the same time the prostate gland of a 63-day-old male was rated as giving a questionable response to similar treatments. When coupled with the following observations — (1) that the prostate gland of the male opossum exhibits precocious development under the influence of relatively small amounts of male hormone, testosterone and testosterone propionate, as early as day 16 (Moore, '41a), and (2) that the prostate gland of a pouch-young opossum made a castrate on day 22 develops in an apparently normal fashion, at least to 50 days of age (Moore, '41b) — the obvious conclusion seems to be that the gonad of the pouch-young male opossum is not normally secreting male hormone, and cannot be stimulated to do so until relatively late in development (questionably at 63 days, and demonstrably at 70 days). Since it is known, however, that in the same animal different accessory sex glands may have different thresholds of response to the same hormone (Hansen, '33; Moore and Gallagher, '30; Price, '36), it was considered desirable to conduct a study of Cowper's glands which had been obtained from gonadotropin-injected opossums.

A. Effects on Cowper's glands in the male

Twelve male opossums aged 17–150 days at the time of autopsy have been available for a study of the effects of equine gonadotropin administration on Cowper's gland. Two commercial preparations, gonadin and gonadogen,² of the gonadotropic principle in pregnant mare serum were employed for daily subcutaneous injection. The earliest treatments began at 10 days and the latest at approximately 136 days. At first a histological study was made in which serial sections of each of the treated glands were compared with serial sections of normal control glands. When it became apparent that consistent histological differences were in evidence, a more objective method was employed

² One rat unit of gonadogen is roughly equivalent to 10 rat units of gonadin.

to determine whether the subjective microscopic analyses were valid. This method consisted of volumetric determinations of the total amount of gland tissue present in glands of four treated and four untreated animals. Each section was projected at 200 magnifications, traced and then measured by means of a planimeter. The volumes are relative rather than absolute.

Since the effects have been found to be fairly consistent, the results will be discussed under two headings: (1) effects up to and including 100 days, and (2) effects at 125 and 150 days.

Effects up to and including 100 days. Opossum 99A received daily injections of 1-rat unit of gonadogen from day 10–16 and was sacrificed on day 17. Cowper's gland anlagen of this treated animal were in a more advanced state of development than that exhibited by the glands of four 16-day controls. The anlagen were more extensively branched, were composed of thicker cords, and were tubulate in portions of the proximal parts. Volumetric determinations disclosed values of 588.5 for the treated and 187.9 for the largest control gland, or more than a three-fold increase in the amount of tissue present. In fact, the volume of tissue was slightly greater than that present in a normal 24-day gland, opossum 10B (table 1). Figures 7 and 4 show the anlagen of the treated 17-day and normal control 16-day animals at the same magnification.

Opossum 102A received the same treatment as the preceding animal from day 10–20, and was preserved on day 21. The anlagen of Cowper's glands were in an advanced stage of morphological differentiation when compared with that which prevailed in the normal 24-day stage. The cords were heavier and more extensively canalized than in the 17-day treated male; moreover, the primordia of Cowper's 1 and 2 were clearly represented. Normally the anlagen do not show signs of hollowing out until day 32. The proximal canalized parts penetrated to within one-cell layer of the sinus canal, but failed to effect an open contact. Very small lumina also appeared in some of the more distal, elongate epithelial cords. Unlike the atypical stimulation effected by androgenic treatment, the gonadotropic effect does not appear to disrupt the normal developmental processes. Volumetric comparisons of the anlagen of this 21-day specimen with those of opossum 10B, the 24-day normal animal, indicated a 48.7% increase.

In opossum 112, a 30-day-old animal treated from day 15–29 with 5-rat units of gonadin, a surprisingly great stimulation of Cowper's gland was noted. Not only do volumetric analyses reveal a 259% increase in the amount of anlagen tissue as compared with that of an

untreated 32-day-old stage, but an almost equivalent amount as compared with the atypically stimulated Cowper's tissue of the androgen-treated 31-day specimen. Figures 10, 12, and 9 show the anlagen of the 30-day gonadotropin-treated animal, a 30-day androgen-treated animal, and a 32-day normal animal. All three lobes are present—Cowper's 3 consists of a number of epithelial strands ventral to the urogenital sinus; Cowper's 2 is also composed of solid cell cords and has slightly luminal ducts; and Cowper's 1 is the largest and most highly developed lobe in respect to duct condition and the number of terminal branchings. The common ducts are patent to the sinus canal, a condition not noted in normal stages prior to day 52. These results are of interest in that gonadotropic treatments which were initiated prior to the early morphological differentiation of Cowper's gland hastened, but did not interfere with the normal developmental processes.

In opossum 196A, treated from day 54–62 with daily injections of 10-rat units of gonadin, the gland was found to be in a state of development comparable to that of the normal 100-day stage. Cowper's 3 was precociously stimulated, and appeared canalized throughout (fig. 6). In a normal male of 75 days Cowper's 3 consists of solid cords (fig. 5); lumina do not appear until day 83. The antero-posterior dimension of Cowper's 3 was found to be 1.18 mm. as compared with 0.64 mm. in the 75-day and 0.77 mm. in the 100-day untreated males. The epithelial cords of Cowper's 1 and 2 were canalized to almost the entire length of their peripheral extensions; the ducts of these parts were large, widely cavitate, and lined with cuboidal to columnar epithelium. The latter was convoluted in some areas. Planimetric determinations again agree in verifying the accuracy of the histological observations. The volume of this 63-day treated gland was found to exceed that of an untreated 65-day male by 125%.

Similar observations to those presented above were made in a 70-day male which had received 10-rat units of gonadin from day 53–69. Cowper's 3 was in a markedly advanced stage of development—almost equivalent, in fact, to that of a normal 125-day gland. The common ducts of Cowper's 1, 2, and 3 were convoluted and partitioned by plicated epithelial strands, which is the condition encountered in the 100-day untreated gland.

In slightly older animals of 80 and 100 days which had received daily injections of gonadotropins for 26 and 15 days respectively, Cowper's glands were in an advanced state of development. Comparatively, the effect of 26 daily treatments of gonadin on Cowper's glands

of animals preserved on day 80, is one of precocious stimulation which results in secretory activity and a state of glandular development surpassing that of the androgen-stimulated 83-day gland and the 150-day untreated gland. While less stimulated than the above, Cowper's glands of the 100-day-old male, sacrificed after 15 daily injections of 5-rat units of gonadogen, were larger in size than the normal 100-day glands. The reason for the limited nature of the response in this animal may be due to the short period of injection. However, inasmuch as it constitutes but one case in five (of animals treated from days 63-100) in which only a slight rather than a marked stimulation has been elicited, it may be regarded as an individual variation from the general trend.

In résumé, the following effects have been observed in Cowper's gland of pouch-young opossums, from 17-100 days, following the injection of gonadotropic hormones. (1) In three animals preserved on days 17, 21, and 30, histological studies and volumetric measurements agree in indicating a precocious stimulation of Cowper's gland primordia. The nature of the effect produced differs from the atypical stimulation which follows androgen treatment in similar-aged animals. (2) In the five older animals preserved on days 63, 70, 80, 80, and 100, there has been pronounced stimulation of Cowper's glands in four instances and slight stimulation in one instance. In each case Cowper's 3, the smallest lobe, shows the greatest degree of stimulation, but pronounced precocious responses are also elicited from Cowper's 1 and 2. The first of these effects, number 1 above, is inconsistent with the studies of Moore ('41a) and Moore and Morgan ('43) on the prostate gland, and will be considered more fully in the Discussion.

Effects of treatments at 125 and 150 days. Two 125-day and two 150-day males were sacrificed after 13 and 14 daily gonadotropic hormone injections, respectively. Such treatment has resulted in a great stimulation of all glandular elements and of secretory activity.

Colloid was present in all tubules and a glandular epithelium which approached the adult type was manifest. The ducts of all lobes were extremely large, widely cavitate, and filled with colloid. The epithelium of Cowper's 1 and 2 was less folded than in Cowper's 3, but the tubules were closely packed with only small amounts of intercellular tissue. Figure 16 shows the condition of the glandular parts in Cowper's 1 and may be compared with figures 19 and 15 which show identically-magnified areas of Cowper's 1 of the 150-day normal and androgen-treated males, respectively.

Comparative measurements of the greatest diameters of Cowper's 1 and 2 and their ducts for androgen-treated, gonadotropin-treated, and normal glands of 150 days are listed in table 3.

Thus, in the 125- and 150-day male opossum the effects of gonadotropic hormone injection on Cowper's glands are very similar, qualitatively, to the responses elicited by similar treatments in certain of the younger stages (70, 80, and 100 days), and closely resemble the stimulating effects produced by androgens in equivalently-aged animals. In these respects the responses of Cowper's glands are similar to the responses of the other male accessory sex gland in the opossum—namely, the prostate.

B. Effects on Bartholin's gland in the female

Bartholin's glands of eleven female opossums, varying in age from 17–150 days, were studied for the possible effects of gonadotropic hormone treatments. The main objectives of this study were, (1) to note any possible histological modifications, and (2) to obtain, if possible, additional data concerning the time at which the opossum ovary can be stimulated to secrete hormones. The duration of treatments and the mode of administration have followed those employed in similar-aged males.

Extended histological and morphological studies have failed to reveal any differences in the epithelial structure or shape of the glands in treated as compared with untreated controls, up to and including 100 days of age. Volumetric studies of three of the earliest stages, 17, 21, and 30 days, failed to reveal any difference between the gland anlagen of treated and of similar-aged normal females. The values are listed in table 2 and may be compared with each other. In six treated animals, ranging in age from 17–70 days, section counts were neither significantly increased nor decreased as compared with those of similar-aged normal specimens. At 80 days, however, the gland of treated opossum 205 extended through 278 sections, that of an 83-day normal gland through 178 sections. Transposed into absolute units, the antero-posterior dimension of the former was 2.78 mm., and that of the latter 1.78 mm. As previously stated no histological differences were apparent. In opossum 90, a 100-day treated animal, the total count was 220, while in two normal 100-day-old animals the count varied from 121 to 171. This represents a smaller antero-posterior dimension of the glands in both treated and normal animals than is encountered in those of the 80-day group, and indicates a variation which serves to cast doubt on the validity of such comparisons, at least in these early stages. Again, no histological differences were apparent between the normal and treated 100-day glands.

In older stages, 125 and 150 days, histological as well as size differences were apparent. The normal 125-day gland is histologically approaching the adult condition. The tubules are close together centrally and more widely separated peripherally. The connective tissue is conspicuous in the peripheral areas, but narrowed into very fine lines centrally where it runs between the high columnar epithelium of the closely bound central tubules. Cellular debris is apparent in the posterior regions of the gland near the point at which the duct leaves the medial aspect, and a homogeneous colloid-like substance is present in some of the lumina. The antero-posterior dimension of the glands in two 125-day normal animals were 2.73 mm. and 2.92 mm. and the greatest dorso-ventral dimensions were 1.62 mm. and 1.35 mm.

In contrast to the condition just described, the histological appearance of Bartholin's glands in two 125-day treated animals differs in certain well-defined essentials. Muscular and connective tissue elements of the gland are tremendously stimulated. The latter, instead of being compact, is very loose — a condition which results in a wide separation of the glandular tubules. The epithelium is low columnar and does not exhibit the folding which occurs in the taller epithelial layers of the normal gland. A homogeneous-staining matrix is present in many of the gland lumina, and distends the ducts to extremely wide, spherical structures in contrast to the smaller, partitioned ducts characteristic of the normal gland. The ducts as well as the posterior-most lumina of the gland are filled with great quantities of cellular debris and leukocytes. The urogenital sinus is likewise filled with cellular debris from the level of the ducts to more posterior regions, and the mucosa is widely cornified. The general picture is one of intense estrogenic stimulation, greater than that which occurs in the normal 125-day animal. The antero-posterior dimension of the gland of opossum 231 was 4.45 mm. (2.73 mm. and 2.92 mm. in the normals), and the greatest dorso-ventral dimension was 2.34 mm. (1.62 mm. and 1.35 mm. in the normals).

A study of Bartholin's gland of a 150-day-old specimen (opossum 164) treated with four daily injections of gonadin, revealed a heightened sloughing of cells and a looser connective tissue stroma than that which occurred in the normal 150-day gland. Due to the shorter period of treatment the effects were not as marked as in the 125-day treated glands. Weights of the ovaries and reproductive tract showed that these structures had been stimulated. The urogenital sinus and the proximal parts of the gland ducts were cornified, a condition not found in the normal 150-day-old animals.

A review of the effects of gonadotropins on Bartholin's gland of the female opossum includes the following salient points. (1) In pouch-young up to 80 days no effects have been observed, either histological or morphological. (2) In 80- and 100-day specimens there appears to be a size increase which is of questionable significance in view of the normal variations, but which suggests a possible early stimulating effect of gonadotropins on the ovary. More experimental studies would of necessity have to be conducted to either validate or void this suggestion. (3) In older animals, aged 125 and 150 days, histological as well as size differences have been observed. The increase in muscular and connective tissue and cellular debris are essentially similar to the effects produced by injected estrogens and are, therefore, the results of increased ovarian activity. The absence of such effects in the adult breeding condition, when, presumably, the ovary is at the peak of estrogenic activity, suggests the possible existence of some other substance which prevents such changes from taking place. In this connection the studies of Price ('41, '42) are very suggestive. This investigator has shown that grafts of the prostate gland of the male and its homologue in the female can be maintained in an active state in adult female hosts, presumably through the action of androgens from luteinized ovaries. The presence of corpora lutea in the adult breeding ovary and of a variable amount of active primary tubules in nearly every adult ovary has been reported by Morgan ('43). This may be the reason for the well developed condition of Bartholin's gland in the adult female opossum.

V. CASTRATION EXPERIMENTS

Through the method developed by Professor Moore, in this laboratory, a number of pouch-young male opossums, the youngest at 22 days of age, have been successfully castrated, and the reproductive tract removed at subsequent stages in development. The writer has been fortunate in having secured the Cowper's glands of these animals for a study of the possible effects of "embryonic" castration. At this stage of the investigation the study is incomplete; therefore, only a portion of the observations can be reported. Although male and female glands have been available for study, this account will deal with only the male tissue. A future communication will consider Cowper's glands of both sexes, and in greater detail.³

Cowper's glands of six male opossums castrated at different periods between days 22 and 40 and sacrificed on days 50, 85, 83, 83, 102, and 102, and four normal untreated controls were studied in serial sections.

³ The results of a more detailed study have already been published (Rubin, '43).

Careful observations have thus far failed to reveal any modifications in the histological or morphological structure of the three lobes of any of the glands. Cowper's 3 of the 102-day-old animal, castrate on day 30, has not deviated in its antero-posterior dimensions or epithelial differentiation from that observed in the 102-day untreated control. The epithelial cords of Cowper's 1 and 2 of this castrate male exhibit the same degree of canalization as that which is evidenced by the same lobes of the control. While it must be admitted that other more precise methods of comparison may bring out certain differences which cannot be analyzed by the relatively crude method employed, the studies at this stage strongly suggest the notion that Cowper's gland of the male opossum undergoes normal morphological and histological differentiation in the complete absence of the gonads — at least to day 102. These studies are in accord with, and lend support to, the hypothesis of Moore ('41a) that reproductive structures differentiate independently of gonad-secreted hormones, at least during the early stages of development.

VI. DISCUSSION

Cowper's glands are characteristic accessory sex glands of the mammalian class and are found in at least some members of each major division. Among the monotremes they are present in the male as a single pair of glands of relatively large size, which open into the ventral wall of the seminal urethra (Owen, 1868; Oudemans, '03; Disselhorst, '04). The presence of the male homologue in the female, *Ornithorhynchus*, was first described by Owen (1841) who called them "Clitorisdrüsen" because of their position to either side of the base of the clitoris.

In marsupials Cowper's glands have been described in many species by a number of workers. While most investigators have agreed that three pairs of Cowper's glands are the usual number to be found in the majority of the male marsupials, there has been a lack of unanimity of opinion concerning a number of species. Thus, Cunningham (1882) and Disselhorst ('04, '04-'08) described a single pair in *Phalangista* (*Trichosurus*); Oudemans ('03) reported two pairs in *Perameles gunni*; Cowper ('04) figured two pairs in *Didelphys* sp.; Leydig (1850) disclosed the presence of four pairs in the unnamed marsupials studied by him; and Fraser ('19) reported the presence of two pairs in *Trichosurus vulpecula*. Contrary to the findings of the earlier workers van den Broek ('10a) expressed the view that all marsupials have three pairs of Cowper's glands, including *Perameles* and members of the *Phalan-*

geridae family. Engle ('26), however, in a review of the male accessory sex glands in vertebrates, accepts Disselhorst's ('04) account, and records the number of Cowper's glands in marsupials as from one to three pairs. In *Didelphys virginiana* recent investigators (Engle, '26; Chase, '39; McCrady, '40) have been unanimous in describing three pairs of Cowper's glands, and the present study agrees with that number.

Studies of Bartholin's glands in marsupials have been fewer in number. Their presence was noted by van den Broek ('10b) who states that "beim Weichen bleibt ein einfacher Zellstrang, welcher nur hin und wieder ein Lumen besitzt, bestehen; aus ihm gehen keine Drüenschläuche hervor." Disselhorst ('04) mentioned the existence of the male homologues in the female phalanger, and Fraser ('19) acknowledges their development in the female of *Trichosurus vulpecula*. The latter investigator, despite the fact that her study failed to encompass the older stages in development, probably followed the lead of van den Broek, and states that "well developed Cowper's glands are present in the male *Trichosurus*. They are also developed in the female, but always remain small and probably undergo reduction in older pouch young." McCrady ('40) describes Bartholin's glands of the adult female opossum, *Didelphys virginiana*, as poorly developed structures on the wall of the urethra.

The findings of the writer in *Didelphys virginiana* fail to agree with any of the previous investigations. Briefly reviewed, Bartholin's glands were found to develop slowly but progressively from the time of their origin to maturity. Peripheral branchings, patent ducts into the urogenital sinus, hollowing out of the solid parts, and the development of a highly differentiated glandular epithelium appeared in a uniform sequence. The glands of the breeding and anestrus adult were found to be well developed and in what appeared to be a functional state. The breeding-season gland with colloid-filled lumina and a well developed vascular supply can hardly be conceived of as a poorly developed, non-functional rudiment. Hence, the present study fails to corroborate the views of either van den Broek or Fraser, although it cannot be denied that a species difference may exist. On the other hand, it should also be emphasized that absolute comparisons of Bartholin's glands in the female and Cowper's glands in the male cannot be made. It is very apparent that an initial difference exists as early as 10 days after birth, when tissue volume measurements reveal a slower rate of development in the female. Obviously then, some factor, probably genetic, is very early in development responsible for a dichotomous type of development

in the two sexes. The female gland never equals the male condition during any stage in development subsequent to the time of origin, but does, however, increase in size and complexity. The growth of Bartholin's gland as a continuous process from the time of origin to maturity differs from the condition of this and other accessories in females of higher mammals. Thus, the female homologue of the prostate gland of the male rat is carried up to a certain state of histological development and then undergoes regression (Price, '36, '39); similar observations have been reported for Bartholin's glands in the rat (Price, '36). The probable role of hormones in effecting the maintenance of the female homologue in the adult opossum has already been elaborated upon in the discussion of gonadotropic effects.

Among placentals Cowper's glands are reported as occurring in some members of practically all of the recognized orders, with the exception of the Sirenians and Cetaceans (Oudemans, '03). In the order Carnivora they are said to be absent in the dog (Leydig, 1850), in the family Mustelidae (Oudemans, '03), and in the members of the suborder Pinnepedia (Oudemans, '03). Variations from the general condition of a single pair of glands have been reported by Rauther ('03) in the hedgehog (*Erinaceus europaeus*) which has two pairs, and in the rodent (*Lepus cuniculus*) which has at least three glands. Descriptions of the female homologue are fewer in number among placentals. Schneidemühl (1880) credits their discovery in the cow to Duverney in 1876 and in the human to Bartholini in 1880. He also states that Cowper, who wrote about Cowper's glands in the human male in 1884, denied the existence of female homologues, although he was subsequently proven wrong. The presence, in the human, of Cowper's gland in the male and a functional homologue in the female is well established. From a comparative point of view the absence of Cowper's glands in certain placentals implies a secondary loss. This presents a condition of especial interest in the aquatic orders where the glands, according to Oudemans, fail to develop in the members of all species.

The present study although not immediately concerned with the still unsettled question of the germ layer origin of Cowper's gland appears to add one or two points of interest which the writer feels should be considered in the light of other investigations. Keibel ('04) and van den Broek ('08, '10a) described the origin of Cowper's glands in monotremes and marsupials, respectively, as outgrowths from the ectodermal cloaca. A similar origin was ascribed to Bartholin's gland in the female marsupial (van de Broek, '10b). Contrary to these findings Buchanan

and Fraser ('18) and Fraser ('19) contend that Cowper's glands anlagen in male and female specimens of the marsupial *Trichosurus vulpecula* arise from the ventro-lateral wall of the urogenital sinus at the point of contact with the cloacal septum (urethral plate), and are, therefore, of endodermal origin. In a text figure, however, Fraser shows Cowper's glands arising as two thickenings of the solid urethral plate, rather than from the wall of the open urogenital sinus. The origin of Cowper's gland in the opossum, *Didelphys virginiana*, is more in accord with Fraser's text figure than with her written description. The finding, already described in detail, that the glands were absent in an adult male lacking a penis adds further confirmation to a urethral plate origin.

In placental mammals the question of the germ layer origin of Cowper's and Bartholin's glands appears equally confused. Hall ('36), Siddiqi ('37), and Barnstein and Mossman ('38) have favored an ectodermal origin in rodents, whereas, an endodermal origin has been suggested by Bissonnette ('24) in the cow, and unequivocally claimed by Felix ('12) in the human. Lichtenberg ('06), however, favors an ectodermal origin in man.

In general it would appear that some solution of this problem might be achieved by determining the germ layer origin of the urethral plate, the structure from which Cowper's glands take their origin. Is this an ectodermal or an endodermal structure? It has already been mentioned that Buchanan and Fraser consider this structure to be endodermal, since, in their studies, the observation was made that the septum originates from the coalesced walls of the endodermal cloaca. Van den Broek ('10a, '10b) also considered this structure to be endodermal. In the opossum the urethral plate is said to originate from the cranial portion of the sagittal cloacal plate (McCrady, '40) which appears during the ninth day of gestation and seems to be of mixed origin, that is, from both ectoderm and endoderm. Since, however, the ectodermal cloaca does not originate until approximately 12 hours before birth (McCrady, '40) it seems likely that the region from which Cowper's glands originate is probably endodermal.

Conflicting viewpoints have arisen amongst investigators of placental mammals. Fleischmann ('02) in a variety of forms, Schwarztrauber ('03) in the sheep and pig, and Felix ('12) in the human consider the urethral plate to be an endodermal derivative, whereas Retterer (1890) in the pig, sheep and rabbit, and Born (1893) in the human designate it as an ectodermal derivative. Barnstein and Mossman ('38) working with the red squirrel, state that the major portion of the urethral plate

is ectodermal and the deep proximal part endodermal. How these varied findings affect the homologies of Cowper's glands in the different mammalian groups may well be questioned, although the discrepancy appears to be one of conflicting interpretation rather than actual difference in origin.

Recently, Burns ('42a) using the estrogenic reaction of tissues as a criterion of the embryological nature of the urogenital sinus of the newborn opossum, has indicated that the urethral plate is ectodermal. Whether this is the result of a secondary conversion by an ectodermal ingrowth subsequent to the original formation of the plate is not clearly indicated. However, the extreme cornification reaction of the sinus epithelium to estrogens reported by Burns, and which prevails to some extent in our material, was not observed in the estrogen-treated anlagen of either Cowper's or Bartholin's glands. In the younger stages stimulation of the epithelial parts was observed, but neither cornification nor desquamation occurred in either the gland or its ducts. In later stages estrogenic effects result in hyperplasia and desquamation, but cornification has not been observed with the exception of the ducts in the region of the cornified sinus.

On the basis of the difference in response to estrogens on the part of Cowper's gland anlagen and the sinus epithelium, two different interpretations may be advanced. Either Cowper's glands are derived from endoderm, which would validate the earlier claims that the urethral plate in marsupials is endodermal, or the estrogen cornification reaction, presumed to be highly specific for the urogenital sinus epithelium and its derivatives, is not applicable to Cowper's and Bartholin's glands of the opossum.

The effects of various hormone treatments on the developing Cowper's and Bartholin's gland anlagen have brought to light certain interesting results which have deviated considerably from the more widely accepted contentions regarding sex hormones and accessory sex gland relationships. It has been demonstrated beyond doubt that the accessory sex glands can be stimulated in young animals by the use of the proper crystalline sex hormones. Thus, Moore and Price ('32) showed that male hormone injections into young male rats resulted in precocious stimulation of the prostate and seminal vesicle. Price ('36) demonstrated that male hormone when injected into either castrate or normal rats either repaired the castrate accessories and stimulated them in some cases above the normal controls, or augmented the normal condition by stimulating histological differentiation and increasing the acinous diameters. The latter investigator has also

demonstrated the ability of the female homologue of the male prostate to maintain a normal histological appearance in the presence of androgenic substances (Price, '41, '42), whereas normally regression occurs at an early age. Wells ('35) has demonstrated that prepuberal ground squirrel accessory sex glands also respond to male hormone by precocious development. In the opossum, the animal with which this paper is concerned, Moore ('39, '41a) has shown that not only does the prostate gland of the male respond precociously, but that the female homologue which never occurs normally can be induced and subsequently stimulated by androgenic treatment. Burns (39a, '39c, '39d) reported similar findings and called attention to the proliferation of the bulbo-urethral (Cowper's) glands.

Contrary to the findings in other mammals and also those concerning the prostate gland in the opossum, androgenic treatment initiated prior to and during the morphological differentiation of Cowper's glands in the male and their homologues in the female has failed to effect a normal type of precocious stimulation. Normal development is interfered with in males up to 39 days, which represents the oldest treated member of the early pouch stages, and in females to considerably later stages. The interference with normal development appears to consist of two opposing responses. One is an inhibition of peripheral branching, in both sexes, and prevention of the early division of the male anlagen into the three lobes which normally occurs at 24–32 days. The other is a marked histological stimulation of the glandular epithelium—such that the total tissue volume is considerably increased over that of the normal gland. This response is, however, limited to younger stages. In prolonged androgen treatments of female animals an underdeveloped histological as well as morphological structure was obtained. A possible explanation of these opposing effects of early androgenic administration is suggested in the observations relating to problems of growth and differentiation. It has been noted in some detail that volumetric analyses reveal a decided increase in the amount of Cowper's and Bartholin's gland tissue in androgen-treated as compared with normal animals. These studies also reveal that in the normal development of Cowper's gland anlagen a fairly rapid growth phase occurs between day 16 and day 24, which is indicated by an increase in volume of approximately three times. Between day 24, when the process of morphological differentiation becomes apparent for the first time, and day 32, when the process is already well established, the rate of volume change is decidedly less than in the previous interval. Comparisons of volumes during similar stages in the androgen-treated

series reveal that the growth rate has been tremendously stimulated. Thus, the 16-day androgen-treated Cowper's anlagen has a volume three times that of the normal 16-day stage, whereas in a 31-day androgen-treated subject the primordia proved to be more than four times the volume of the equivalent structure in the 32-day normal male. Although it cannot be definitely ascertained that such is actually the case, these studies appear to correlate rather well with the thesis that "there is some antagonism between differentiation and growth" (Weiss, '39). The same general suggestion can be applied to the effects obtained with estrogens which paralleled those produced by androgens. Foote ('41) has noted similar developmental trends in the Müllerian ducts of estrogen-treated salamanders. This investigator found that primary development was inhibited, whereas the histological and functional differentiation of the ducts was stimulated.

The effects of crystalline androgens on the early development of Cowper's gland likewise raises the question of whether embryonic hormones similar to the injected male hormones (which in adult animals give every indication of being the true male hormone) are being produced within the normally developing embryo. Two observations would seem to indicate that a negative reply to this question is in order: (1) injections of male hormone during early stages in development have failed to elicit a normal type of precocious response from Cowper's gland anlagen, and (2) injections of male hormone in later stages of development, 70-80 days, appear to stimulate the glands to a state which simulates advanced stages in normal development.

In view of the effects produced by injections of androgens and estrogens, the results of gonadotropin injection serve to raise several pertinent questions concerning the nature of embryonic humoral influences. For purposes of review it may be noted that gonadotropic substances have evoked precocious responses from Cowper's gland during all of the developmental stages which have been studied. In the early stages of development the type of response differed markedly from the atypical stimulation, which followed androgen treatment. Normal development was hastened rather than interfered with, as revealed by histological inspection and volumetric measurements. In later stages the precocious responses were entirely similar to those resulting from androgen treatment. In females no response could be detected in Bartholin's gland prior to 80 days, but at this stage a suggestion of a slight stimulating effect was observed.

Previous investigations in the opossum seemed to indicate that the male gonad was incapable of responding to gonadotropic injections

prior to day 63; similarly, the female gonad, while exhibiting certain histological effects, was considered to be refractory prior to day 125 (Moore and Morgan, '43). The indicators used were the prostate gland in the male, and the uterus and lateral vaginal canals in the female, structures which respond in an apparently normal fashion to small amounts of pure androgens and estrogens, respectively. In other mammalian forms (Smith and Engle, '27; Wells, '35; Price, '36) and in birds (Domm, '37) gonadotropic substances have been effective in stimulating precocious hormone secretion in young subjects. In this respect Cowper's glands of the opossum seem to indicate that gonadotropins exert an effect earlier in the life of the animal than heretofore suspected. Why should this be apparent from a study of Cowper's and not from a study of the prostate? The suggestion has already been advanced that a threshold may exist for the different opossum accessory sex glands similar to the one known to exist in the rat. Thus, the prostate gland, which has been used as an indicator by Moore ('41a; and Moore and Morgan, '43) may have a higher threshold of response, and, therefore, require a greater amount of circulating hormone to effect a noticeable change in its histological structure than does Cowper's gland. The validity of this suggestion can, perhaps, be experimentally tested by using smaller dosages of androgens to determine whether or not Cowper's gland can be stimulated without at the same time stimulating the prostate gland. The recent findings of Burns ('42b) that small doses of androgens when injected into pouch-young opossums can selectively stimulate certain portions of the reproductive tract without affecting others is highly suggestive. Even should this prove to be true, it would still remain to be shown that Cowper's glands can be precociously stimulated by androgens in a manner comparable to that which results upon the injection of gonadotropins. The observations made thus far indicate that only atypical stimulation can be obtained with the androgens employed. In females, treated with gonadotropins, there has been a suggestion of an effect on Bartholin's glands by the 80th day. Morgan ('43) and Moore and Morgan ('43) have failed to obtain definite indications of ovarian secretion prior to day 125. Further observations of a more consistent nature will be required to determine with certainty whether Bartholin's gland of pouch-young animals can be used as an accurate indicator of ovarian activity.

A question of major importance arises from the considerations just presented. If the opossum testes, as indicated by Cowper's gland, can be made to secrete hormones at an early stage in development, does this necessarily imply that they do so in the absence of gonadotropic stimu-

lation? The effects of castration appear to supply a partial answer to this question. In a limited number of male animals castrate at an early age it has been determined that the histological and morphological differentiation of Cowper's glands has proceeded normally to the latest stage studied—102 days. Thus, in the complete absence of the gonads the gland has shown a capacity for continued growth and differentiation. This implies that such growth and development are not dependent on testis-secreted hormones. The question may then be raised as to whether Cowper's gland maintenance depends on hormone secretion during any stage in the life of the animal. This question may be answered by pointing to the fact that a number of investigators have demonstrated that Cowper's glands of various adult mammals are dependent upon testis-secreted hormone for normal maintenance (the opossum: Chase, '39; the rat and guinea pig: Heller, '30, '32, Barrington, '14; the ground squirrel: Wells, '35; the rabbit: Leydolph, '29; the bull: Schneidemühl, 1883; the pig: Baker, '28). Studies now in progress should indicate at what stage in the development of the opossum such dependence becomes manifest.

One other item should be mentioned briefly, and that is the possibility that extra-gonadal hormones may be instrumental in maintaining the normal histological state of the accessory sex glands in castrate animals. Thus, the experiments of Price ('36, '39, '42), Howard ('38), Davidson and Moon ('36), and Burrill and Greene ('39) indicate that the adrenals secrete androgenic substances which are capable of maintaining the histological state of the prostate gland in castrate male rats for at least the first 6 or 7 weeks of post-natal development. The production of such androgenic substances in the pouch-young opossum is still, however, devoid of proof. Until such time as it is definitely known whether or not they occur, it seems more tenable to ascribe early growth and differentiation of Cowper's gland to the same genetic and possibly humoral forces postulated by Moore ('41a) to be the effective agents in sex differentiation.

VII: SUMMARY AND CONCLUSIONS

NORMAL DEVELOPMENT

1. In the opossum, *Didelphys virginiana*, Cowper's gland anlage in the male and its homologue (Bartholin's gland anlage) in the female appear for the first time at 5 days after birth, as paired, bilateral, solid thickenings of the proximal end of the sagittal urethral plate.

2. The male anlagen undergo a much more rapid type of development, and by 32 days are already branching into the three definitive parts

of the adult gland, Cowper's 1, 2, and 3. The female anlagen remain close to the ventro-lateral wall of the urogenital sinus, continue to grow, but fail to branch in the manner described for the male. Thus, a distinct dichotomous type of development is apparent in very early stages.

3. In the male a single pair of common ducts open in the ventro-lateral aspect of the urogenital sinus canal by the 52nd day. In the female the paired, bilateral ducts open into the sinus canal by the 67th day.

4. Local differentiation in Cowper's gland proceeds at a uniform rate, lumina develop slowly from proximal to more distal parts, and the embryonic epithelium gradually acquires a glandular appearance. These changes appear at an earlier stage in the larger lobes, but between day 83-100 rapid differentiation occurs in the smallest lobe, so that it appears to be more advanced than either Cowper's 1 or 2.

5. Evidence obtained from histological study of the normal stages indicates that male hormone secretion in the opossum may be initiated prior to day 125.

6. Bartholin's gland of the female opossum does not regress at any period in development. Changes occur in the histological structure of the normal gland which indicate ovarian activity by day 125.

7. On the basis of position, mode of development, and histological structure, it seems probable that the entire female gland corresponds to the small, paired lobes in the male designated as Cowper's 3.

8. The adult glands of the male are highly developed structures with colloid-filled lumina and large glandular ducts. Histologically the lobes differ slightly from one another. Each is enclosed in a striated muscle capsule, as are also the individual and common ducts. In the female the gland of the breeding and anestrus season are well developed, the former somewhat better than the latter. Secretory activity is evidenced by the presence of colloid within the large, glandular, epithelial-lined lumina. The suggestion has been made that male hormone probably produced by the ovary is the factor which maintains the gland in the female.

HORMONE TREATMENTS

Sex hormones

1. Pure chemical androgens and estrogens have been found to exert a similar effect on the early primordia of Cowper's gland in the male and Bartholin's gland in the female. This effect has been described as a general stimulation of growth, precocious canalization of the cords, and interference with the processes of normal development.

2. In no case have androgens been effective in stimulating male development in the female homologue, whereas estrogens have not suppressed the formation of Cowper's gland anlagen in either sex.

3. The nature of the early responses, especially to androgens, has suggested the possibility that if circulating hormones are present in the pouch-young opossum, they are probably unlike the crystalline substances designated as male hormones.

4. Prolonged treatments with either androgens or estrogens in young females have resulted in marked inhibition of morphological development. A certain degree of recovery has been noted upon the cessation of such treatments.

5. In older males, subsequent to 70 days, androgens have produced typical precocious responses simulating advanced stages in normal development. In females, subsequent to 100 days, androgens are effective in producing a better developed glandular epithelium than that which normally prevails.

6. In males, subsequent to day 70, but prior to day 150, injected estrogens have produced hyperplasia and desquamation in Cowper's glands and hyalinization of the proximal duct epithelium, which is in agreement with similarly reported effects on the accessory sex glands of other mammals. At 150 days the dosages applied were insufficient to noticeably affect the glandular epithelium, but hyperplasia of connective tissue and muscle was evidenced. In 150-day-old females the process of desquamation, which is characteristic of the normal gland of this age, is accelerated to some extent. In an anestrus adult female estrogens effected epithelial desquamation, whereas normally it is absent or only slightly manifest. An explanation has been advanced to explain this result.

Gonadotropic hormones

1. The effect of gonadotropic hormones on the growth of Cowper's glands seems to indicate that the opossum testis can be precociously stimulated at an early stage in development.

2. The difference in the type of response resulting from gonadotropins and injected androgens seems to support the notion that if there are hormones produced by the pouch-young animal's own tissues they are unlike the synthetic male hormones.

3. A threshold effect has been suggested to explain the positive response of Cowper's gland and the absence of a response in the prostate gland to the same treatments.

4. In females gonadotropins have been ineffective prior to 80 days. Subsequent to this period some suggestion of a response is detected. This may indicate precocious ovarian stimulation before 100 days.

5. In older stages, subsequent to 100 days, marked testicular and ovarian stimulation are evidenced in the responses of Cowper's and Bartholin's glands. The similarity of administered and gonad-secreted androgens becomes evident at this time.

6. The possibility that gonadotropins administered during the early stages in development are stimulating organs other than the gonad has not been ruled out.

CASTRATION EXPERIMENTS

1. Six pouch-young male opossums made castrate at an early age, in some cases preceding the morphogenesis of Cowper's gland, were found to have well differentiated, normal-appearing Cowper's glands up to 102 days, which represents the latest stage studied.

2. These observations indicate that the testes and hence, testis hormones, are not essential for the normal histological and morphological differentiation of Cowper's gland.

3. Other suggestions concerning extra-gonadal influences have been discussed.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Normal 5-day-old female (1A), showing Bartholin's gland anlagen as bilateral thickenings arising from the urethral plate. Similar origin of Cowper's gland occurs in 5-day-old male. Uro-proctodeum and anal glands below. 50 X.

2 Normal 8-day-old male (3), showing Cowper's gland anlagen to either side of urethral plate. 50 X.

3 Normal 32-day-old female (34A). Bartholin's gland anlagen ventrolateral to smooth muscle of the urogenital sinus. 50 X.

4 Normal 16-day old male (33A). Cowper's gland anlagen, as solid cords extending dorso-laterally from the lower urogenital sinus epithelium. 50 X.

5 Normal 75-day-old male (79A), showing solid epithelial parts of Cowper's 3 below the urogenital sinus, and the slightly canalized parts of Cowper's 2 to the right of the sinus. 22 X.

6 Gonadotropin-treated 63-day-old male (196A), showing the precociously hollowed parts of Cowper's 3 in the crescent-shaped stroma below the urogenital sinus. Compare with fig. 5. 22 X.

7 Gonadotropin-treated 17-day-old male (99A). Precocious canalization of Cowper's gland anlagen in the region of the urogenital sinus. Compare with fig. 4. 50 X.

8 Normal 52-day-old male (41), showing canalized Cowper's gland ducts lateral to the urogenital sinus, the oblong-shaped mesenchymal capsules and partially tubulate epithelial cords of Cowper's 2 to either side of the rectum (above), and the crescent-shaped duct of Cowper's 1 below and medial to Cowper's 2 on the right. 22 X.

9 Normal 32-day-old male (34B). Cowper's 2 on the left and the slightly canalized duct of Cowper's 1 on the right. 50 X.

10 Gonadotropin-treated 30-day-old male (112), showing the precociously canalized condition of the ducts, Cowper's 2 on the left, and Cowper's 1 on the right. Urogenital sinus below, rectum above. 50 X.

11 Estrogen-treated 31-day-old male (39A), showing precocious canalization and histological differentiation of Cowper's gland anlagen. Note lack of branching, exudate in lumina, and cornification of urogenital sinus epithelium. 50 X.

12 Androgen-treated 30-day-old male (101A), showing precocious canalization and histological differentiation, but lack of branching characteristic of normal Cowper's anlagen. (fig. 9). Urogenital sinus epithelium stimulated (no cornification). 50 X.

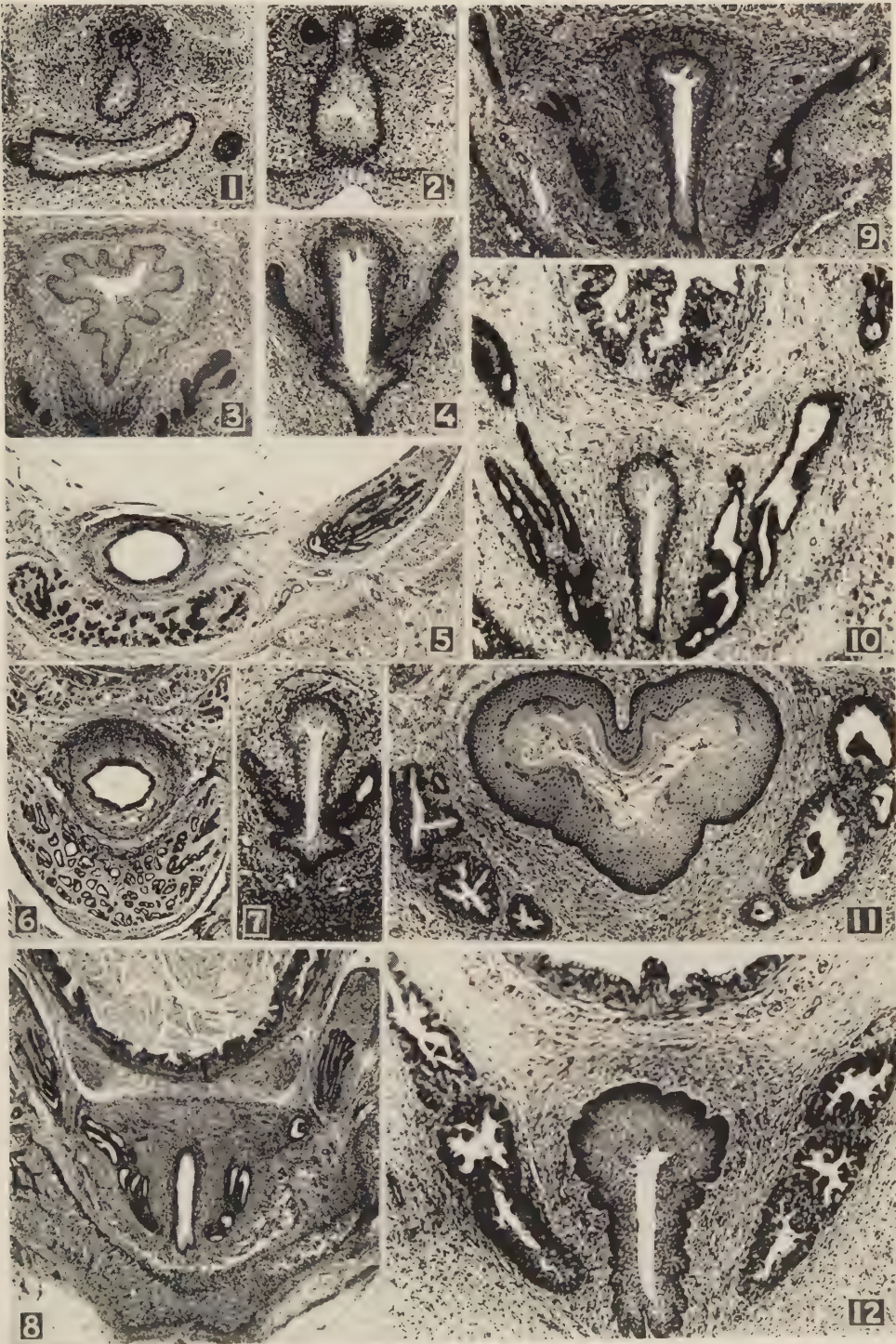


PLATE 2

EXPLANATION OF FIGURES

- 13 Normal 100-day-old female (82B), showing Bartholin's gland and accessory lobes in lower right and left hand corners. 22 $\times$.
- 14 Androgen-treated 150-day-old male (172). Cowper's 3. 340 $\times$.
- 15 Androgen-treated 150-day-old male (172). Cowper's 1. 340 $\times$.
- 16 Gonadotropin-treated 125-day-old male (226). Cowper's 1. Note colloid filled lumina. 340 $\times$.
- 17 Androgen-treated 95-day-old female (118), showing the under-developed condition of Bartholin's gland. Compare with fig. 13. 22 $\times$.
- 18 Normal 150-day-old male (177). Cowper's 3. 340 $\times$.
- 19 Normal 150-day-old male (177). Cowper's 1. 340 $\times$.

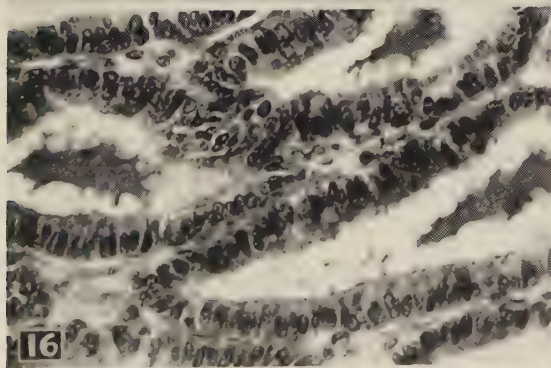
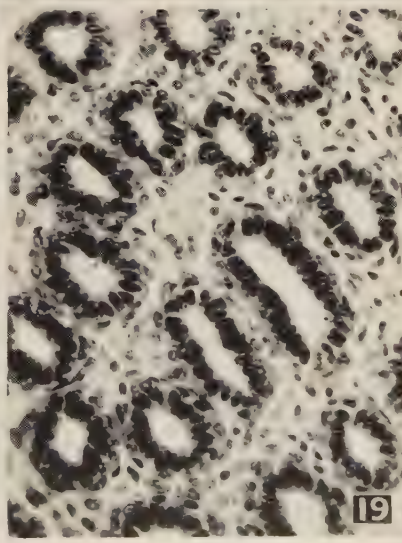
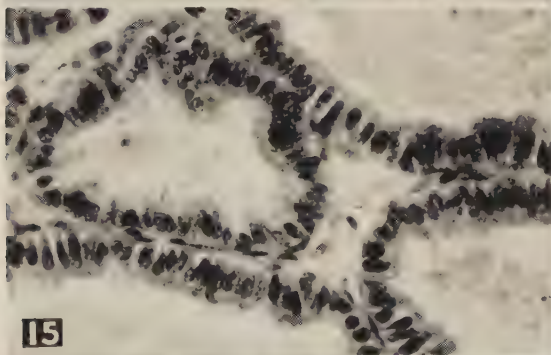
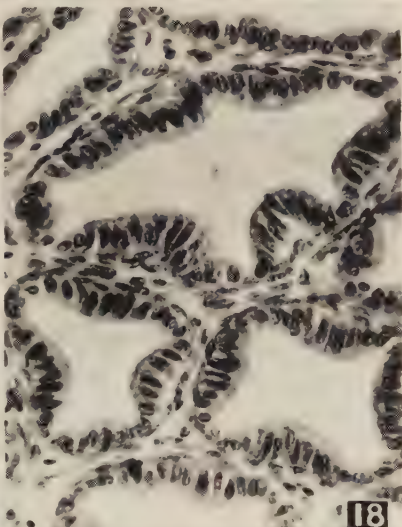
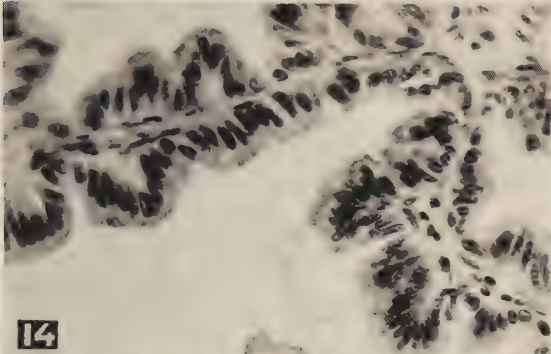
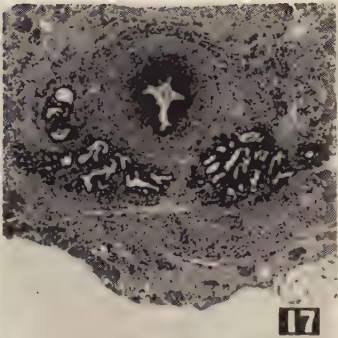
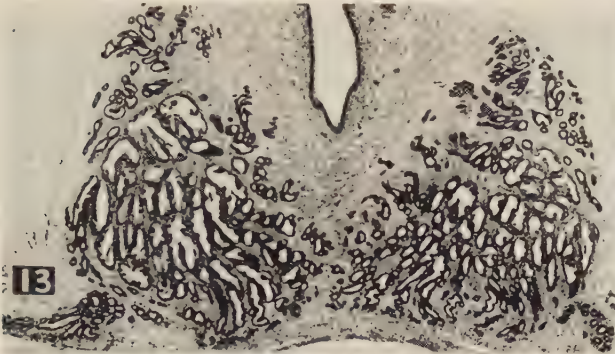


PLATE 3

EXPLANATION OF FIGURES

20 Normal 150-day-old female (179). A section through the confluent area of the bilateral Bartholin's glands. Note the cellular debris in the large centrally situated lumina. Urogenital sinus in the center. 22 $\times$.

21 Androgen-treated 150-day-old female (175), showing one-half of Bartholin's gland to the right of the urogenital sinus. In comparing with fig. 20 note the closely packed lumina, the lack of cellular debris, the presence of colloid in some of the lumina, and the large laterally extending accessory lobules on the lower right. A portion of urogenital sinus is visible in the upper left hand corner. 22 $\times$.

22 Bartholin's gland of the breeding adult female in May. High columnar epithelium, secretion in lumina. 340 $\times$.

23 Bartholin's gland of the anestrus female in September. High columnar epithelium, small amounts of cellular debris in lumina. 340 $\times$.

24 Bartholin's gland of an estrogen-treated anestrus female in September. Increased amount of connective tissue, lowered epithelium, and great quantities of cellular debris in lumina. 340 $\times$.

